



**HAL**  
open science

# The genetics of intraspecific plant-plant cooperation in *Arabidopsis thaliana*

Harihar Jaishree Subrahmaniam

► **To cite this version:**

Harihar Jaishree Subrahmaniam. The genetics of intraspecific plant-plant cooperation in *Arabidopsis thaliana*. Life Sciences [q-bio]. Université Toulouse III Paul Sabatier, 2020. English. ⟨NNT : ⟩. ⟨tel-02531785⟩

**HAL Id: tel-02531785**

**<https://hal.science/tel-02531785v1>**

Submitted on 3 Apr 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization



# THÈSE

## En vue de l'obtention du DOCTORAT DE L'UNIVERSITÉ DE TOULOUSE

Délivré par l'Université Toulouse 3 - Paul Sabatier

---

Présentée et soutenue par  
**Jaishree SUBRAHMANIAM**

Le 16 mars 2020

**Identification des bases génétiques de la coopération plante-  
plante chez la plante modèle Arabidopsis thaliana**

---

Ecole doctorale : **SEVAB - Sciences Ecologiques, Vétérinaires, Agronomiques et  
Bioingenieries**

Spécialité : **Ecologie, biodiversité et évolution**

Unité de recherche :

**LIPM - Laboratoire des Interactions Plantes-Microorganismes**

Thèse dirigée par

**Fabrice ROUX et Dominique ROBY**

Jury

**Mme Irene Till-Bottraud**, Rapporteur

**Mme Bodil Ehlers**, Rapporteur

**M. Jerome Enjalbert**, Examineur

**M. Christophe Thébaud**, Examineur

**M. Fabrice ROUX**, Directeur de thèse

**Mme Dominique ROBY**, Co-directrice de thèse

*The genetics of intraspecific plant-plant  
cooperation in Arabidopsis thaliana*

# Acknowledgments

*“Nothing of significance was ever achieved by an individual acting alone”*

*John C. Maxwell*

The work presented in this thesis would not have been possible without the cooperation of many people. I take this opportunity to extend my sincere gratitude and appreciation to everyone who made this PhD thesis possible.

I am indebted to my supervisors, Fabrice Roux and Dominique Roby for the faith they put in me by giving me an opportunity to chase my dreams. Thank you for your guidance throughout this journey and for inspiring me, in your different ways, towards the path of science. Your enthusiasm, passion for research, and mission for providing high-quality work, has made a deep impression on me. I am grateful to have had a chance to learn from both of you.

I would also like to express my sincere gratitude to my thesis committee members, Etienne-Pascal Journet and Céline Richard-Molard for guiding me through the course of my research project.

To the past and current members of ECOGEN team, thank you for the cooperative links we formed during my time here. The timely help you all gave me and the friendships we formed over these three years will be cherished forever. Thank you all for your continual patience, support and warm humor for making my PhD journey a fun-filled one!

I also would like to take this opportunity to thank all the members of the greenhouse staff of LIPM for their constant love and support during my PhD. Thank you for everything!

To every friend I made here at LIPM, you all made this journey a whole lot easier, entertaining and enjoyable. Merci beaucoup for all the pep-talks, unlimited cups of caffeine, hugs, sound advice, encouragements and kicks-up-the-backside and mostly for all the love you gave me!

To all my friends back home, even though you were not physically here, thank you for rooting for me and for believing I could do this, even when I didn't.

Kummu and Toppai, Tachi, Subulu, Shanku, Zizu, Abloo, and Adi, this one's for all of you!

## Abstract

Despite the importance of plant-plant interactions in the functioning of both natural ecosystems and agro-ecosystems, studies on plant-plant interactions still have two major gaps to be addressed, that is (i) the genetics of natural variation of plant-plant interactions and (ii) the relative importance of positive interactions within plant species. After writing two reviews on the state-of-the-art related to these gaps, I decided to understand the adaptive genetic bases of intraspecific positive plant-plant interactions in *Arabidopsis thaliana* at two geographical scales. To do so, I adopted an interdisciplinary approach between quantitative genetics, ecology and genome-wide association mapping. Firstly, based on a field experiment designed to study natural variation of genotype-by-genotype interactions among 52 whole-genome sequenced natural populations from the Midi-Pyrénées region, I identified two different strategies of positive interactions, *i.e.* kin cooperation and overyielding. Natural variation of positive interactions among these populations were mainly associated with biotic ecological factors varying at a fine spatial scale, such as presence of commensal bacteria or Shannon index of plant communities in the native habitats. Importantly, QTLs associated with variation of positive interactions were significantly enriched in genomic signatures of local adaptation. Secondly, based on a greenhouse experiment using 195 whole-genome sequenced accessions collected in a local French population located in a highly diverse and competitive environment, we revealed the existence of certain genotypic combinations that were benefitting reciprocally to each other's presence, resulting in a 'super overyielding' strategy. Importantly, genetic dissimilarity at the QTLs associated with this strategy was detected for such pairs, supporting the 'compatibility genes' hypothesis as underlying this 'super overyielding' strategy. Finally, at both geographical scales, we detected a predominance of metabolism related gene functions underlying natural variation of positive interactions, which might be explained by their putative roles in (i) recruitment of similar microbiota by kin to explain kin cooperation, and (ii) potential complementary metabolite cross-feeding to explain overyielding. The next step is undoubtedly cloning of the candidate genes to identify causal associations, thereby allowing to start getting a glimpse on the genetic and molecular landscape associated with positive interactions in *A. thaliana*.

**Keywords:** intraspecific variation, genotype-by-genotype interactions, cooperation with reciprocal benefit, kin cooperation, overyielding, local adaptation, selective ecological agents, association genetics.

## Résumé

Malgré l'importance des interactions plante-plante dans le fonctionnement des écosystèmes naturels et des agro-écosystèmes, les études sur les interactions plante-plante ont encore deux lacunes importantes à combler, à savoir (i) la génétique associée à la variation naturelle des interactions plante-plante et (ii) l'importance relative des interactions positives au sein des espèces végétales. Après avoir rédigé deux revues faisant un état de l'art sur chacune de ces lacunes, j'ai décidé de m'intéresser à l'étude des bases génétiques adaptatives des interactions plante-plante positives chez *Arabidopsis thaliana* à deux échelles géographiques. Pour cela, j'ai adopté une approche interdisciplinaire entre génétique quantitative, écologie et génétique d'association pangénomique. Dans un premier temps, sur la base d'une expérience réalisée sur un terrain expérimental conçue pour étudier la variation naturelle des interactions génotype-génotype entre 52 populations naturelles de la région Midi-Pyrénées, j'ai identifié deux stratégies contrastées d'interactions positives, à savoir la coopération entre apparentés (kin cooperation) et la surproduction (overyielding). La variation naturelle des interactions positives entre ces populations était principalement associée à des facteurs écologiques biotiques variant à une fine échelle spatiale, tels que la présence de bactéries commensales ou l'indice de Shannon des communautés végétales dans les habitats natifs. Par ailleurs, les QTL associés à la variation des interactions positives sont significativement enrichis en signatures génomiques d'adaptation locale. Dans un deuxième temps, à partir d'une expérience en serre basée sur 195 accessions collectées dans une population locale française située dans une communauté végétale très diversifiée et compétitive, nous avons révélé l'existence de certaines combinaisons génotypiques où chaque accession bénéficie réciproquement de la présence de l'autre accession, résultant en une stratégie de 'super overyielding'. De manière intéressante, nous avons trouvé que les accessions coopératives étaient génétiquement très différenciées au niveau des QTL associés à cette stratégie, ce qui soutient l'hypothèse des 'gènes de compatibilité' comme étant à la base de cette stratégie de 'super overyielding'. Finalement, aux deux échelles géographiques, nous avons identifié que la variation naturelle des interactions positives étaient associées à une prédominance de fonctions génétiques liées au métabolisme, ce qui pourrait s'expliquer par leurs rôles potentiels dans (i) le recrutement de microbiotes similaires par deux plantes ayant le même génotype pour expliquer la coopération entre apparentés, et (ii) l'alimentation croisée complémentaire de métabolites pour expliquer la surproduction. La prochaine étape est sans aucun doute le clonage des gènes candidats pour identifier les associations causales, ce qui pourrait permettre de commencer à avoir un aperçu du paysage génétique et moléculaire associé aux interactions positives chez *A. thaliana*.

**Mots clés:** variation intraspécifique, interactions génotype-génotype, coopération avec bénéfiques réciproques, kin cooperation, overyielding, adaptation locale, agents écologiques sélectifs, génétique d'association.

# Contents

<b>Introduction.....</b>	<b>8</b>
I. Importance of plant-plant interactions.....	9
I.A. General overview.....	9
I.B. The genetics underlying natural variation of plant-plant interactions, a beloved but forgotten member of the family of biotic interactions.....	12
I.C Towards unifying evolutionary ecology and genomics to understand genotype-by-genotype interactions within wild plant species.....	37
I.D. Prevailing questions in the study of intraspecific interactions?.....	82
II. <i>Arabidopsis thaliana</i> as a model species to unravel the adaptive genetic and molecular bases of plant-plant interactions?.....	82
II.A. General characteristics.....	82
II.B. Genetic and genomic resources and tools available for <i>Arabidopsis thaliana</i> .....	83
II.C. A model for studying natural variation of plant-plant interactions?.....	86
III. How to identify the genetic basis of adaptation?.....	87
III.A. Genome wide association (GWA) mapping.....	89
III.B. Genome-Environment Association (GEA).....	91
IV. Outline of the thesis.....	93
V. References.....	95
<b>Chapter 1 .....</b>	<b>100</b>
<b>Establishing a genomic map of local adaptive cooperation in <i>Arabidopsis thaliana</i> .....</b>	<b>100</b>
I. Introduction.....	101
II. Objective.....	103
III. Materials and methods.....	103
III.A. Biological material.....	103
III.B. Experimental design.....	105
III.C. Phenotypic trait measurement.....	106
III.D. Statistical analysis of natural variation of positive interactions.....	107
III.E. GWA mapping using a Bayesian hierarchical model.....	110
III.F. Testing for signatures of local adaptation.....	113
III.G. Estimating putative ecological drivers of positive interactions.....	114
IV. Results.....	115
IV.A. Natural genetic variation of positive GxG interactions.....	115
IV.B. Genetic bases of positive GxG interactions and their adaptive status.....	118
IV.C. Selective ecological drivers of positive GxG interactions.....	120

IV.D. Genetic bases of local adaptive cooperation in 52 local populations.....	124
V. Discussion and perspectives.....	126
V.A. Natural genetic variation of intraspecific positive interactions.....	126
V.B. Genetic bases of local adaptive positive interactions.....	128
V.C. Selective ecological drivers of intraspecific positive interactions.....	130
V.D. Candidate genes underlying the identified QTLs: pre-eminence of functions related to metabolism.....	132
VI. References.....	135
VII. Supplementary information.....	141
<b>Chapter 2.....</b>	<b>149</b>
<b>Investigating positive GxG interactions at the genomic level in a local population of <i>Arabidopsis thaliana</i>.....</b>	<b>149</b>
I. Introduction.....	150
II. Objective.....	153
III. Materials and methods.....	153
III.A. Biological material.....	153
III.B. Experimental design.....	155
III.C. Phenotypic trait measurement.....	156
III.D. Statistical analysis of natural variation of positive interactions.....	157
III.E. GWA mapping combined with a local score analysis.....	160
III.F. Estimating genetic relationships underlying the ‘super overyielding’ strategy.....	160
IV. Results.....	161
IV.A. Natural genetic variation of GxG interactions within a local population.....	161
IV.B. Genetic architecture of positive GxG interactions.....	167
IV.C. Investigating relationships between cooperators at the genomic level.....	169
IV.D. Genetic bases associated with the ‘super overyielding’ strategy.....	171
V. Discussion and perspectives.....	171
V.A. The occurrence of a ‘super overyielding’ strategy in the local TOU-A population.....	171
V.B. The ‘super overyielding’ strategy observed in the TOU-A population may be driven by compatibility genes.....	172
V.C. Candidate genes underlying the ‘super overyielding’ strategy.....	173
VI. References.....	175
VII. Supplementary information.....	178
<b>General discussion and perspectives.....</b>	<b>184</b>
I. Introduction.....	185

II. Natural genetic variation of intraspecific positive interactions at different geographic scales .....	189
III. Biotic factors as the main selective drivers of positive interactions? .....	191
IV. What are the molecular determinants of cooperative plant-plant interactions?.....	193
V. References .....	194
VI. Supplementary information .....	195

## *Introduction*

*“...it may be argued, therefore, that the essential qualities that determine the ecology of a species may only be detected by studying the reaction of its individuals to their neighbours”*

*John Harper, 1964*

## *I. Importance of plant-plant interactions*

### **I.A. General overview**

During their life cycle, individuals face a multitude of stresses, from abiotic or biotic origins. This is especially true for plants, because of their sessile way of life. In the context of current global changes such as climate change, urbanization, habitat fragmentation, a profound change in these stresses is to be expected in terms of both their intensity and their nature. A major challenge in evolutionary ecology is therefore to understand and predict the ability of a plant species to persist in the presence of new environmental conditions.

However, in nature, plants are seldom found alone. Be it meadows, backyard gardens or forests, plants can be easily seen to be in direct or indirect relationships with a large number of plants of the same as well as different species. Plant–plant interactions play an important role in regulating plant communities and ultimately ecosystems through their effects on resource availability and habitat structure (Brooker 2006). Studying the mechanisms underlying plant-plant interactions is therefore essential to understand the functioning of communities, which may in turn help to predict ecosystem responses to global change (Martorell & Freckleton 2014).

For several decades, it has been recognized that plant-plant interactions play a major role in the structure, diversity and dynamics of plant communities (Tilman 1985, Goldberg & Barton 1992, Chesson 2000). These interactions between plants can range from competitive (- -) to reciprocal helping (++) through commensal (+0) and asymmetrical relationships (+-) (Subrahmaniam *et al.* 2018). Among these four main types of interactions between plants, competition has been the most widely studied and results in negative impact on growth and fitness on both plant partners (Keddy 2015). Since all plants share a few basic requirements, limitations of resources such as the availability of nutrients, water or light could drive competition between plants (Turkington & Harper 1979, Chaney & Baucom 2014). In contrast, positive interactions between individuals (mutualism between individuals of different species and cooperation between individuals of the same species) has recently regained attention for their role in regulating plant community composition and assembly, as well as to elucidate overyielding in crop mixtures

## *General introduction*

(Bertness & Callaway, 1994, Callaway 1995, Brooker & Callaghan, 1998, Bruno *et al.* 2003, Wendling *et al.* 2017).

Despite their importance in many of the key mechanisms involved in the functioning of both natural ecosystems and agro-ecosystems, two major gaps still have to be addressed in the study of plant-plant interactions. Firstly, the genetic and molecular determinants that underlie the natural variation in plant-plant interactions are still poorly understood. Identification of the genetic bases of plant-plant interactions is of supreme importance for both crops and wild plant species. For example, in crops, detection and characterization of QTLs underlying enhanced crop competitive ability, weed suppressive ability, or more recently overyielding can help in accelerating crop breeding programs (Subrahmaniam *et al.* 2018). On the other hand, in wild plant species, understanding the genetic bases underlying plant-plant interactions could be essential for predicting the adaptive potential of natural plant communities (Pierik *et al.* 2013), especially to face current anthropogenic modifications of habitats (Frachon *et al.* 2019). Secondly, natural plant communities can be disintegrated into assemblages of different populations of different species. In addition, multiple genotypes of a given species constitute a plant population. These local genotypes are bound to interact either directly due to their physical proximity, or indirectly through volatile cues or by soil conditioning during inter-cohort interactions. An under-appreciated aspect of community assembly framework is that of intraspecific genotype-by-genotype (GxG) interactions within a population. Moreover, positive interactions are also expected to be prominent within a population and not among individuals coming from different populations, owing to their repeated, frequent interactions (Nowak 2006). In addition, positive interactions have been postulated to be selected under stressful environments at the interspecific level (Bertness & Callaway 1994). But to date, attempts to establish patterns of interactions within wild plant species have led to equivocal results for existence of positive interactions between genotypes of a species (File *et al.* 2012).

Therefore, to start addressing these two main gaps in the study of plant-plant interactions, we decided to write two reviews to report the state of the art about (i) the genetics of plant-plant interactions and (ii) the relative importance of intraspecific GxG interactions, in particular positive interactions, in wild plant species. To have a glimpse of the genetic and molecular mechanisms underlying plant-plant interactions that have been identified so far, we first reviewed 63 studies (including studies on global change in gene expression as well as QTL and GWA studies) that

## *General introduction*

aimed to characterize these mechanisms when a plant is directly challenged by another plant. In the second review, we decided to make a synthesis on intraspecific GxG interactions within wild herbaceous plant species based on 66 articles published in the last 35 years. We summarized the current state of affairs centered around positive interactions (*i.e.*, kin cooperation and overyielding), and also highlighted the need to unify evolutionary ecology and genomics to obtain a thorough picture of the patterns of GxG interactions within wild species.

**I.B. The genetics underlying natural variation of plant-plant interactions,  
a beloved but forgotten member of the family of biotic interactions**

## SI PLANT BIOTIC INTERACTIONS

# The genetics underlying natural variation of plant–plant interactions, a beloved but forgotten member of the family of biotic interactions

Harihar Jaishree Subrahmaniam<sup>1</sup>, Cyril Libourel<sup>1</sup>, Etienne-Pascal Journet<sup>1,2</sup>, Jean-Benoît Morel<sup>3</sup>, Stéphane Muñoz<sup>1</sup>, Andreas Niebel<sup>1</sup>, Sylvain Raffaele<sup>1</sup> and Fabrice Roux<sup>1,\*</sup>

<sup>1</sup>LIPM, Université de Toulouse, INRA, CNRS, Castanet-Tolosan, France,

<sup>2</sup>AGIR, Université de Toulouse, INRA, INPT, INP-EI PURPAN, Castanet-Tolosan, France, and

<sup>3</sup>BGPI, INRA, CIRAD, SupAgro, Université de Montpellier, Montpellier, France

Received 18 October 2017; revised 2 December 2017; accepted 6 December 2017; published online 12 December 2017.

\*For correspondence (e-mail [fabrice.roux@inra.fr](mailto:fabrice.roux@inra.fr)).

## SUMMARY

Despite the importance of plant–plant interactions on crop yield and plant community dynamics, our understanding of the genetic and molecular bases underlying natural variation of plant–plant interactions is largely limited in comparison with other types of biotic interactions. By listing 63 quantitative trait loci (QTL) mapping and global gene expression studies based on plants directly challenged by other plants, we explored whether the genetic architecture and the function of the candidate genes underlying natural plant–plant interactions depend on the type of interactions between two plants (competition versus commensalism versus reciprocal helping versus asymmetry). The 16 transcriptomic studies are unevenly distributed between competitive interactions ( $n = 12$ ) and asymmetric interactions ( $n = 4$ , all focusing on response to parasitic plants). By contrast, 17 and 30 QTL studies were identified for competitive interactions and asymmetric interactions (either weed suppressive ability or response to parasitic plants), respectively. Surprisingly, no studies have been carried out on the identification of genetic and molecular bases underlying natural variation in positive interactions. The candidate genes underlying natural plant–plant interactions can be classified into seven categories of plant function that have been identified in artificial environments simulating plant–plant interactions either frequently (photosynthesis, hormones), only recently (cell wall modification and degradation, defense pathways against pathogens) or rarely (ABC transporters, histone modification and meristem identity/life history traits). Finally, we introduce several avenues that need to be explored in the future to obtain a thorough understanding of the genetic and molecular bases underlying plant–plant interactions within the context of realistic community complexity.

**Keywords:** allelopathy, altruism, competition, cooperation, diffuse biotic interactions, gene expression, GWA mapping, mutualism, parasitic plant, QTL mapping.

## INTRODUCTION

Throughout its life cycle, a plant can interact simultaneously and sequentially – directly or indirectly – with many plant neighbors, whether in crop fields or in more natural environments (Williams, 2013). In such plant networks, a large diversity of interactions can be observed both at the intraspecific and interspecific levels (Box 1). Intraspecific relationships extend from competition with conspecifics (same species) to cooperation, through altruism (Box 1)

(Dudley, 2015). Interspecific relationships include competition with heterospecifics (different species), reciprocal helping (i.e. mutually beneficial interactions), commensalism (i.e. facilitation) and asymmetric interactions such as parasitism and allelopathy (Box 1).

Surprisingly, despite: (i) the importance of plant–plant interactions in mediating plant community structure, diversity and dynamics (Tilman, 1985; Goldberg and Barton,

**Box 1** Terminology of the various categories of plant–plant interactions

According to Dudley (2015), interactions between plant individuals can be divided into various categories based on whether they occur between two species (heterospecific or interspecific interactions) or within a species (conspecific or intraspecific interactions).

Interaction	Nature	Interspecific level	Intraspecific level
Competitive	--	Competition	Competition
Commensal	+0	Facilitation	Cooperation with direct benefit
Reciprocal helping	++	Mutualism (co-adaptation)	Cooperation with reciprocal benefit
Asymmetric	+–	Parasitism	Altruism
		Allelopathy	

The interaction is termed competitive (--) when both interacting individuals suffer significant cost by investing in competing and therefore compromising on the benefit. In other words, the outcome of competition for both the interacting individual plants can be viewed as Benefit < 0 and Cost > 0. The terms benefit and cost are pertaining to the net effect on individual fitness of both the interacting individuals. A popular example for interspecific competition is the interaction between many crops and weeds which leads to a significant reduction in agricultural crop yield as the weeds compete for resources that would otherwise be available for the crops to use.

Commensal interactions (+0) are the ones in which the helper plant provides benefit to another plant but does not incur any cost in the process. It can be represented as Benefit > 0, Cost = 0 for individual X, the one receiving the help and Benefit = 0, Cost = 0 for individual Y, the one providing the help. This kind of interaction is called facilitation when it occurs at interspecific level whereas at intraspecific level, it is called 'cooperation with direct benefit'. At the interspecific level for example, epiphytes that grow on the barks of many trees purely for physical support are good examples for this type of interaction. The host tree does not incur any cost in providing anchorage to the epiphyte and the epiphyte can cling on to the host plants without being parasitic and damaging the host plant organs or functions.

Reciprocal helping (++) is the interaction where both the partners exchange costly help. For both the interacting individuals, the cost of providing help is significant but it is compensated for by the benefit they get in return, i.e. Benefit > Cost for both interacting individuals. This reciprocation is directed to only specific individuals that would return the favor. It is called mutualism when it occurs between species and 'cooperation with reciprocal benefit' when it is within a species. Mutualism is thought of as a result of co-adaptation and both the interacting individuals affect the evolution of the helping trait phenotype of each other. Teste *et al.* (2014) conducted an experiment where they grew four plants species having different nutrient acquiring strategies under nutrient rich and poor conditions. They observed that under nutrient poor conditions, the focal plant *Melaleuca preissiana* (arbuscular mycorrhizal/ectomycorrhizal fungal network) grew better when it was grown besides *Eucalyptus marignata* (EM fungal network) and *Banksia menziesii* (cluster mining roots) in a mesh microcosm where roots were not in physical contact but only the fungal network were mingling. The plants were able to acquire nutrients and share them between neighbours depending on the nutrient acquiring strategy of the neighbour and using the fungal network under limited soil resources. This experiment is evidence that plants can be involved in reciprocal helping but only when there is a need for them to share benefits.

Asymmetric interactions (+–) occur when one of the interacting partners benefits at the expense of the other (Halvy *et al.*, 2017). This 'costly' help can be depicted as Benefit > 0 and Cost = 0 for the individual receiving the help and Benefit = 0 and Cost > 0 for the help provider. Parasitism and allelopathy come under this category at the interspecific level (NB: few studies also reported allelopathy at the intraspecific level such as in *Kalanchoe daigremontiana*; Groner, 1974). Parasitic plants like *Arceuthobium* sp. that derive nutrition from other plants and causing harm to the host are prime examples for this interaction at the interspecific level. Some plants release inhibitory chemicals, allelochemicals (juglone, sorgelone etc.) via their roots that can affect the development and growth of neighboring plants. Although allelopathy includes both positive (growth promoting) and negative (growth inhibiting) effects, definitions of allelopathy often only consider negative effects (Olofsdotter *et al.*, 2002). The interaction between allelopathic plants and their neighbors is therefore considered as asymmetric. At the intraspecific level, it is related to 'altruism' that corresponds to the preferential help given to an individual from the same population without getting any direct benefit for it. Individuals should perform actions that increase their own fitness but altruism is quite the opposite of that. Individuals that perform altruistic actions reduce their own chances of reproduction and survival in order to help another.

Altruism evolves within a population where individuals provide costly help to their relatives. Helping a relative is selfish in some sense as it increases the fitness of the altruist indirectly. Relatives that share a significant portion of genes between themselves and if the altruist decides to help a relative, that means more chance of representation of its own genes in the next generation. This nepotistic behavior of individuals within a population is called kin recognition. Help can also be provided if the actor recognizes a gene / set of closely linked genes and only favors the carrier of those genes. This preferential help based on genetic similarity only at some parts of the genome is called Greenbeard effect. But, to date, there have been no report about the existence of Greenbeard genes in plants. The defining feature of kin recognition is based on the concept of inclusive fitness, a concept that has been popularly used to describe the evolution of eusociality among many animal species. However, this view has recently been debated by many theoretical and experimental studies (Nowak *et al.*, 2010; Allen *et al.*, 2013), claiming that a group can begin to cooperate even if individuals are unrelated, providing the association proves useful to both parties. This association can be a product of reciprocity or mutualistic synergism (Nowak *et al.*, 2010).

1992; Chesson, 2000); and (ii) weeds having a significantly higher average potential to reduce crop yield than any other crop pest (34% for weeds versus 18% for animal pests versus 16% for pathogens; Basu *et al.*, 2004; Oerke, 2006; Neve *et al.*, 2009), our understanding of the genetic and molecular bases underlying natural variation of plant–plant interactions is largely limited in comparison with other types of biotic interactions. For example, among the 56 genes functionally validated for being associated with natural variation in response to biotic interactions in the model plant *Arabidopsis thaliana*, more than one-third confer resistance to herbivory while the rest of the genes are more-or-less evenly distributed among interactions with viruses, bacteria, fungi and oomycetes (Roux and Bergelson, 2016). The only gene identified as involved in plant–plant interactions underlies responses to root spatial constraints (used as proxy for thigmotropic responses to other plants within the rhizosphere) and not the direct response to a neighbor plant (Joseph *et al.*, 2015). In addition, in early 2017, 35 Genome-Wide Association studies (GWAS) reported the fine mapping of genomic regions associated with natural variation of plant response (either crops or natural species) to pathogen infection (Bartoli and Roux, 2017), whereas only one GWAS reported the identification of QTLs underlying plant–plant interactions (Baron *et al.*, 2015).

Deciphering the genetic and molecular bases underlying natural variation of plant–plant interactions can however be fundamental to propose new germplasm management strategies for maintaining sustainable provision of yield and other ecosystem services in an agro-ecological context (Litrice and Violle, 2015). For example, the identification of genetic markers usable in Marker-Assisted Selection (MAS) can largely accelerate breeding programs to address several major agro-ecological issues. Firstly, weeds are farmers' worst pests, especially in organic systems (Basu *et al.*, 2004; Neve *et al.*, 2009; Asif *et al.*, 2015). In addition, an increase of the deleterious impact of weeds on crop yield due to climate change is expected (Clements *et al.*, 2014; Peters *et al.*, 2014). QTLs associated with increased competitiveness in crops can therefore represent a durable and sustainable alternative for weed control (Worthington and Reberg-Horton, 2013). The genetics of competitiveness can be based on the detection and functional characterization of QTLs underlying enhanced crop competitive ability (such as traits linked to plant canopy establishment and nutrient acquisition capacity; Olofsdotter *et al.*, 2002), or QTLs underlying weed suppressive ability through the production of chemical defense compounds (such as allelopathy; Khanh *et al.*, 2007). Secondly, during the last decades, a particular attention from breeders has been devoted to improving yield per unit of field area by increasing plant density (Guo *et al.*, 2011), where the

target of the breeding programs is population and/or community performance (i.e. group selection) rather than individual plant performance (i.e. individual selection) (Weiner *et al.*, 2010). Because the deleterious effects of a large range of abiotic (i.e. drought stress) and biotic (i.e. pathogen attack) stresses are exacerbated in high-density conditions (Gonzalo *et al.*, 2010; Ku *et al.*, 2016), there is a need for identifying the genetic basis underlying density-related stress tolerance (Gonzalo *et al.*, 2006). Thirdly, increasing species diversity and/or genotypic diversity has positive effects on plant productivity, stability of yield and ecosystem services (Tilman 1997; Tilman *et al.*, 2001; Crutsinger *et al.*, 2006; Johnson *et al.*, 2006; Isbell *et al.*, 2011; Loreau and de Mazancourt, 2013; Prieto *et al.*, 2015). Therefore, in the framework of resource-use complementarity, understanding the effects of trait combinations involved in interactions and their underlying genetics, between a focal plant and neighboring conspecific and/or heterospecific plants, may help to optimize species assemblages in cropping systems (e.g. intercropping systems) (Litrice and Violle, 2015; Pakeman *et al.*, 2015). It will undoubtedly accelerate breeding programs aimed at creating elite mixtures also called 'ideomixes' (Litrice and Violle, 2015).

Identifying and characterizing the function of genes associated with natural variation of plant–plant interactions is also fundamental to predict and understand adaptive dynamics and evolutionary trajectories of natural plant communities (Pierik *et al.*, 2013). Understanding the genetic bases and modes of adaptation underlying plant–plant interactions in current plant communities is essential to accurately estimate responses of a plant species to ongoing drivers of global change (Roux and Bergelson, 2016). In particular, it can help estimate the potential of plant species to face anthropogenic modifications of plant assemblages, which may result from differences of geographic range shift among native species under climate change (Bachelet *et al.*, 2001; Gilman *et al.*, 2010; Singer *et al.*, 2013) or from increased plant biomass and reduced diversity under climate warming (Baldwin *et al.*, 2014). Furthermore, intraspecific diversity can largely contribute to biotic resistance to exotic invasion, as illustrated by intraspecific diversity in the dominant North American native *Pseudoroegneria spicata* improving resistance against the strong exotic invader *Centaurea stoebe* (Yang *et al.*, 2017). Identifying the genetic bases associated with natural variation of suppressive ability against invasive species may strongly help to propose management strategies, such as reinforcing invaded native populations by planting native individuals with the allelic combination that limits or suppresses invasion. Finally, because genetic diversity within plant populations can be strongly associated with species diversity in interacting communities they support (such as arthropod communities; Whitham *et al.*,

2006), this relationship may have important conservation implications. For example, the maintenance of genetic diversity of an endangered species can be dependent on the level of genetic diversity of the associated native plant species, thereby leading to the concept of minimum viable interaction population (Whitham *et al.*, 2006). To date, genetic diversity of plant populations has been traditionally estimated based on genetic markers that are expected to behave neutrally. Identifying the plant genetic bases associated with natural variation of associated community phenotypes may increase the power of designing appropriate management strategies to maintain endangered species.

Here, we review the genetics and molecular mechanisms underlying plant–plant interactions. We first provide an overview of the main molecular mechanisms underlying the perception of the signals related to the presence of neighboring plants and how these signals are translated into response strategies. While very informative, most of these molecular mechanisms have been initially identified in artificial environments designed to simulate plant–plant interactions. Therefore, in a complementary way, we then list studies based on plants directly challenged by other plants. In particular, because QTL mapping and global gene expression studies are approaches well adapted to interrogate genes mediating biotic interactions in a systematic manner, we reviewed the QTL mapping studies reporting the genetics underlying natural variation of plant–plant interactions and the studies reporting global change in gene expression underlying natural interactions within and between species. We particularly explore whether the genetic architecture and the function of the candidate genes underlying natural plant–plant interactions depend on the type of interactions between two plants (conspecific versus heterospecific, competition versus commensalism versus reciprocal helping versus asymmetry). We also emphasize cases in which gene functions in plant–plant interactions differ between artificial and ecologically relevant conditions. Finally, we introduce several avenues that need to be explored in the future to obtain a thorough understanding of the genetic and molecular bases underlying plant–plant interactions within the context of realistic community complexity.

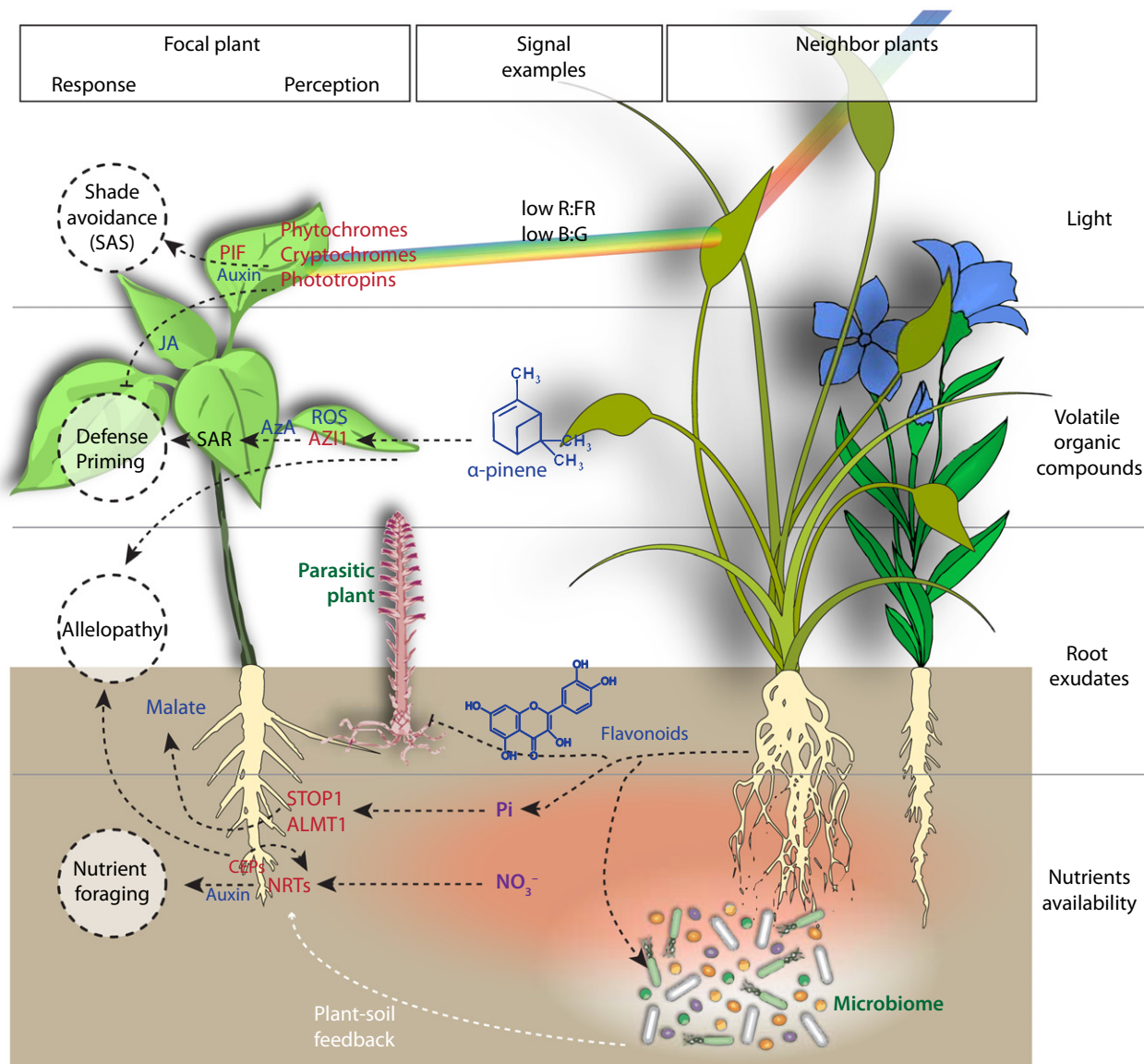
## NEIGHBOUR DETECTION AND RESPONSE STRATEGIES

Focal plants have the ability to perceive the nature and intensity of the interactions with neighboring plants through diverse signals, transmitted either above or below ground (reviewed in Pierik *et al.*, 2013; Gundel *et al.*, 2014). These signals can be classified as: (i) indirect signals, corresponding to environmental factors modified by the presence of neighbors, such as light and nutrients; and (ii) direct signals, corresponding to molecules directly

produced by neighboring plants, such as aerial volatile organic compounds (VOCs) and soluble root exudates. Recent work has led to the identification of novel genes and molecules mediating signals between plants, and improved our understanding on how the signals emitted by neighboring plants are integrated into an optimal response strategy. We review here the progress from the last 3 years on these points (Figure 1).

### Light signal and shade avoidance syndrome

Due to absorbance of specific light wavelengths by chlorophyll, neighboring plants alter the quality and/or quantity of light perceived by the focal plant, triggering an escape strategy designated as the shade avoidance syndrome (SAS) (reviewed in Ballaré and Pierik, 2017). In crowded environments, the ratio of red/far-red light (R:FR) and blue/green light (B:G) are strongly reduced. These changes in light quality are perceived by phytochromes and cryptochromes respectively, through signaling pathways converging to the Phytochrome Interacting Factors (PIF) that integrate multiple light cues and adjust the SAS response (Fraser *et al.*, 2016; de Wit *et al.*, 2017). Interestingly, this light decoding system has been recently proposed to discriminate between kin and other neighbors, triggering altruistic or cooperative SAS (Crepy and Casal, 2015; but see Till-Bottraud and de Villedemereuil, 2016). SAS involves the elongation of hypocotyls, stems and petioles, and the upward re-positioning of certain leaves (i.e. hyponasty) and requires biosynthesis of the auxin phytohormone (de Wit *et al.*, 2016). In *A. thaliana*, auxin fluxes have been recently shown to mediate local organ-specific responses in the focal plant to heterogeneous light signals originating from the surrounding plants (Michaud *et al.*, 2017; Pantazopoulou *et al.*, 2017). Using focal leaf illumination with low R:FR ratio, the authors showed that leaf tips, but not petioles, were sensitive to R:FR reduction, leading to a hyponastic response in the treated leaf only. In addition, global transcriptome and mutant analyses revealed the increased expression of auxin biosynthesis and auxin efflux carrier genes in response to low R:FR ratio at the leaf tip. Local exogenous auxin application and R:FR treatment on plants expressing auxin-reporter constructs indicated that auxin is transported from the leaf blade to the petiole to cause hyponasty. Mathematical leaf models showed *in silico* that perception and response to light signals in distinct leaf areas should increase growth in densely populated environments (Pantazopoulou *et al.*, 2017). Low R:FR ratio in densely planted sunflower fields leads to alternate stem inclinations, which is required for increased oil production per land area, thereby illustrating the adaptive value of neighbor detection in cropping systems (López Pereira *et al.*, 2017). Low R:FR ratio also modulates the expression of jasmonic acid (JA)-mediated immunity



**Figure 1.** Neighbor detection and response strategies in plant–plant interactions.

The main classes of signals and clues that mediate plant–plant communication are indicated on the right hand side of the figure: light, aerial volatile organic compounds (VOCs), root exudates and nutrient availability. Examples of signals of each class and components of the corresponding response mechanism in the focal plant discussed in the text are shown. Low ratios of red/far-red light (R:FR) or blue/green light (B:G) light are signals associated with neighbor plants. Response to these signals involve notably phytochromes, cryptochromes, phototropins, PIF proteins, auxin and jasmonic acid (JA) hormones. A typical response is the shade avoidance syndrome (SAS). VOCs include for instance  $\alpha$ -pinene and  $\beta$ -pinene that mediate plant–plant interactions via AZI1 protein, reactive oxygen species (ROS) and the systemic signal azelaic acid (AzA). They trigger systemic acquired resistance (SAR) and defense priming. Root exudates include flavonoids (structure shows quercetin) and malate. Some of these compounds act on parasitic plants, on bacteria of the soil microbiome or on the availability of nutrients. Plant–plant interactions mediated by exudates are often designated as allelopathy and can result in alterations of the plant biomass allocation. The availability of nutrients such as Pi and  $\text{NO}_3^-$  is sensed by plant proteins such as STOP1, ALMT1 or NRTs, triggering the systemic movement of CEPs and release of auxin and malate notably. Plant peptides and proteins are labeled in dark red; plant metabolites are labeled in blue; black arrows show positive connections; black bar-headed lines show negative connections; some connections between elements of the figure have been omitted for clarity.

genes in a species-specific manner (Gommers *et al.*, 2017). JA signaling seems to be required for phytochrome B (i.e. the predominant phytochrome controlling SAS in response to low R:FR ratio) to repress plant immunity but not to trigger SAS under low R:FR ratio (Cerrudo *et al.*, 2017). Direct physical contact between leaf tips is able to

induce leaf hyponasty in *A. thaliana* (de Wit *et al.*, 2012). Such mechanosensing is also connected to defense priming via JA (Chehab *et al.*, 2012; Mbengue *et al.*, 2016), pointing towards a strong connection between physiological responses of the focal plant to its neighbor plants and pathogens.

### Nutrients uptake and foraging

Uptake by roots of neighboring plants creates a heterogeneous nutrient and water environment for the focal plant that triggers morphological and physiological responses designated as foraging. These responses mainly correspond to the modulation of root distribution and architecture to increase nutrient uptake and the modulation of transport of nutrients and systemic signal across the plant (Aibara and Miwa, 2014). For example, nitrate sensing and transport by NRT1.1 lead to the repression of lateral root elongation in low nitrate conditions through the activation of the ANR1 transcription factor and modulation of auxin traffic (O'Brien *et al.*, 2017). Upon nitrogen depletion, roots secrete small C-terminally Encoded Peptides (CEPs) which are translocated to the shoot and are perceived by leucine-rich repeat receptor kinases (LRR-RKs) to activate nitrate transporters such as NRT1.1 (Sun *et al.*, 2017 for a review; Tabata *et al.*, 2014). Recent additions to the list of foraging regulators include STOP1 and ALMT1 that mediate phosphate-induced root remodeling through malate exudation (Balzergue *et al.*, 2017; Mora-Macias *et al.*, 2017). Depending on the source of phosphate available in the soil, the grass *Deschampsia cespitosa* produces more biomass when grown with a different grass species than with conspecifics, suggesting that nutrient availability regulates plant competition (Ahmad-Ramli *et al.*, 2013). In addition, growing on soil previously occupied by diverse plant genotypes increased nitrogen uptake in roots of *D. cespitosa* compared with soil conditioned by siblings (Semchenko *et al.*, 2017) and a role of soil microbiome was suggested.

### Microbiome and other intermediates

The impact of soil microbiome on plant growth and responses to stress is being increasingly appreciated (Berendsen *et al.*, 2012; Lebeis *et al.*, 2015; Vandenkoornhuyse *et al.*, 2015). Microbes can also act as intermediates in plant–plant exchanges, such as in the case of mycorrhizal networks connecting roots of several plants (reviewed in Selosse *et al.*, 2006). Nutrient exchanges through mycorrhizal networks can be highly asymmetric and may strongly favor the growth of some plant species over the others (Walder *et al.*, 2012). More generally, the impact of soil microbes on the dynamics of plant communities is designated as plant–soil feedback (PSF) (Bever *et al.*, 2011; Van de Putten *et al.*, 2013). Two recent studies conducted on Mediterranean shrublands and temperate forests showed that plant diversity can be negatively impacted by soil pathogens (negative PSF) but also positively impacted by neighboring plants with distinct associations of symbiotic microbes for nutrient acquisition (positive PSF) (Bennett *et al.*, 2017; Teste *et al.*, 2017). In these studies, the strong protection against pathogens conferred by ectomycorrhizal fungi reduced plant diversity

in favor of their host species, whereas arbuscular mycorrhizal fungi led to the establishment of more diverse plant species. Reciprocally, soil suppressiveness against the fungal pathogen *Rhizoctonia solani* increased with species diversity in artificial plant communities (Latz *et al.*, 2015, 2016). This is consistent with the information that the soils of permanent species-rich grasslands harbor a more diverse microbiome and are more suppressive against soil-borne fungal pathogens than cultivated land (Garbeva *et al.*, 2006).

### Root exudates

Nutrient and water availability, soil microbiome and small molecules released by plant roots (root exudates) form an interconnected network of below-ground signals affecting plant–plant interactions (Bais *et al.*, 2006). Root exudates include a large diversity of molecules that are often species specific and vary depending on the above-ground and below-ground environment (reviewed in van Dam and Bouwmeester, 2016; Massalha *et al.*, 2017). Root exudates also vary significantly at the intraspecific level. For example, some *A. thaliana* accessions lack an indolic glucosinolate hydrolysis product or a hydroxycinnamic acid conjugate, due to specific disruptive mutations affecting genes of the corresponding biosynthetic pathways (Mönchgesang *et al.*, 2016). This very specific molecular signature is consistent with the idea that soluble root exudates could contribute to the ability of plants to recognize individuals of the same genotype from others (reviewed in Depuydt, 2014). For example, rice roots were shown to grow towards roots of plants from the same genotype, but away from roots of plants from different genotype (Fang *et al.*, 2013). Similarly, the growth of *D. cespitosa* roots differed when treated with root exudates collected from plants of the same or different genotypes (Semchenko *et al.*, 2014).

Allelopathy is defined as the effect(s) of one plant on other plants through the release of chemical compounds in the environment (Rice, 1984; Olofsdotter *et al.*, 2002). Among chemical compounds, root-exuded allelochemicals, such as sorgoleone, that have negative growth effect on neighbor plants, are of primary importance to improve overall competitive ability of many crops (rice, wheat, barley, oat, sorghum...) against weeds (Olofsdotter *et al.*, 2002; Jensen *et al.*, 2008). Root-exuded allelochemicals produced by a plant can also have positive effects on other plants. Therefore, there has been a growing interest for the possible exploitation of these positive effects on plant growth in agricultural systems through intercropping (Brooker *et al.*, 2015). In maize–faba bean intercrops, maize root exudates were shown to promote flavonoid synthesis in faba bean, along with an increase of nodulation and nitrogen fixation (Li *et al.*, 2016). Reciprocally, faba bean root exudates increased maize growth via facilitating

increased phosphorus availability in the soil (Zhang *et al.*, 2016). In this system, rhizobia that associate with faba bean root to fix nitrogen are important intermediates from the soil microbiome. Root exudates also play a key role in the interaction of crops with parasitic plants. Resistance to *Striga* parasitic plants in sorghum cultivars was found to result from a change of the dominant strigolactone 5-deoxystrigol (a highly active *Striga* germination stimulant) in root exudates to orobanchol, another strigolactone compound that does not stimulate *Striga* germination (Gobena *et al.*, 2017). *Desdemonium* plant species produce C-glycosylflavonoid in their root exudates that inhibit *Striga* parasitism on maize, making them useful intercrop species in some small-holder farms (Hooper *et al.*, 2015).

### Volatile organic compounds

In response to endogenous or exogenous signals, plants can produce very diverse VOCs. VOCs released in response to herbivore attack, such phenolics, alkaloids, terpenes, are well known to induce defense priming, conditioning stronger and faster subsequent defense responses (Baldwin *et al.*, 2006; Dicke and Baldwin, 2010). Owing to their long distance effect, VOCs can attract insect predators to prey-attacked plants (Schnee *et al.*, 2006), and they are exploited by some plant pathogens to attract pollinators and favor their dispersal (e.g. Roy, 1993). Recent work in *Petunia* flowers demonstrated that VOC emission can be mediated by its active transport across the plasma membrane. This emission could prevent toxic accumulation of VOCs in plant cells and increase the reach of emitted compounds (Adebesin *et al.*, 2017). Although various plant tissues can emit VOCs, most studies reporting plant–plant interactions mediated by VOCs involve airborne green leaf volatiles. For example, VOCs produced by damaged sagebrush plants protect neighboring *Nicotiana attenuata* plants from herbivores (Karban *et al.*, 2014). Soft mechanical stimulation also triggers VOC emission protecting plants from herbivores (Markovic *et al.*, 2016). VOCs emitted by undamaged neighboring plant can also trigger changes in biomass allocation between shoots and roots in focal plants (Ninkovic, 2003). In response to VOCs from heterospecific undamaged plants, potato plants modified the composition of their emitted VOC cocktail and were less frequently visited by aphids (Ninkovic *et al.*, 2013). By genetically manipulating VOC emissions in *N. attenuata* plants, Schuman *et al.* (2015) showed that the protective effects on the focal plant were dependent on the degree of herbivore infestation, while loss of protection in VOC-deficient plants was consistently compensated by neighboring plants. This suggests that targeted alterations in the VOC metabolism of a few plants could provide community-level protection in fields (Schuman *et al.*, 2015). By contrast, damage to a neighbor plant decreased protection against herbivores in the field for plants that were close relatives

(Pearse *et al.*, 2012), highlighting the complexity of VOCs-mediated plant resistance in realistic environments (Baldwin *et al.*, 2006). During systemic acquired resistance (SAR), *A. thaliana* plants release  $\alpha$ -pinene and  $\beta$ -pinene VOCs, which in turn can elicit SAR in distal plants and protect them against the bacterial pathogen *Pseudomonas syringae* pv. *tomato*, through the induction of reactive oxygen species (ROS) accumulation (Riedlmeier *et al.*, 2017). This function requires the activity of the putative lipid-transfer protein AZI1 that stimulates the systemic movement of the SAR signal azelaic acid (AzA) (Cecchini *et al.*, 2015; Riedlmeier *et al.*, 2017), possibly connecting plant–plant communication and the integration of plant defense signals in the focal plant.

### THE GENETICS OF NATURAL VARIATION OF PLANT–PLANT INTERACTIONS

To obtain a complementary picture of the molecular bases underlying plant–plant interactions identified up to date, we examined studies where focal plants have been directly challenged by neighbor plants. Therefore, we have not considered studies performed in artificial environments designed to simulate plant–plant interactions, such as shade (Nagatani *et al.*, 1993; Reed *et al.*, 1993, 1994) or root spatial constraint (Joseph *et al.*, 2015). Although simulated environments are highly powerful to decipher the molecular mechanisms underlying the perception of a particular signal (Gundel *et al.*, 2014; Ballaré and Pierik, 2017), it does not embrace the range and complexity of signals that are perceived by a focal plant directly interacting with a neighbor plant (Moriles *et al.*, 2012; Horvath *et al.*, 2015; Figure 1). Neither did we include studies focused on phenotypic traits thought to be involved in plant–plant interactions, such as improved seedling establishment and early growth measured in absence of plant–plant interactions (Addisu *et al.*, 2009). In addition, we did not cover either association studies or transcriptomic studies based on a restricted number of genes. We instead focused on studies reporting whole-genome scans. Based on these criteria, we identified a total of 63 studies reporting the identification of QTLs and/or candidate genes underlying natural plant–plant interactions (Figure 2 and Data Set S1).

### Highlights on the nature of the data

Despite the limited number of studies that we identified, important observations have to be drawn before extracting trends on the genetic and molecular bases underlying natural plant–plant interactions. Firstly, as exemplified in *A. thaliana*, screening EMS mutants has been a widely adopted approach to start tracking down the molecular mechanisms underlying interactions with various pathogen species, in particular viruses and bacteria (Roux and Bergelson, 2016). However, although several EMS mutants initially identified in environments simulating plant–plant

Approach <sup>a</sup>	Type of interaction	Focal species <sup>b</sup>	Neighbor-species/Treatment	CR <sup>c</sup> CE <sup>d</sup> Plant organ <sup>d</sup>	Growth cond. <sup>e</sup> Arch. <sup>f</sup>	Candidate genes <sup>g</sup>	Reference <sup>h</sup>
Global change in gene expression	competition conspecific	<i>Arabidopsis thaliana</i>	intra-genotypic competition vs inter-genotypic competition	ne ne roots	C	UP: ABC transporter, pathogen response, metabolism, cytochrome	Biedrzycki et al., 2011
		<i>Arabidopsis thaliana</i>	intra-genotypic competition (four density conditions)	ne ne shoot and leaves	C	UP: photosynthesis, ABC transporter, metal ion transporter	Geisler et al., 2012
		<i>Arabidopsis thaliana</i>	absence and presence of intra-genotypic competition at multiple densities	ne ne rosette leaves and roots	C	DOWN: regulation of active oxygen species, defense response DOWN in low density but UP in high density: biotic and abiotic stress perception	Masclaux et al., 2012
		<i>Hordeum vulgare</i> (4 genotypes)	intra-genotypic competition (two density conditions)	ne ne leaves	C	UP (leaves + roots): nutrient starvation, biotic and abiotic stress response, defense response UP (leaves): genes expressed during low R:FR ratio	St. Pierre et al., 2011
		<i>Trifolium lucidum</i>	absence and presence of inter-genotypic competition	ne ne roots	C	UP: photosystem, auxin, DOWN: histones	Bowsher et al., 2017
		<i>Zea mays</i> (5 genotypes)	intra-genotypic competition (two density conditions)	ne ne leaves	C	DOWN: disease resistance UP: auxin, photosystem	St. Pierre et al., 2011
		<i>Zea mays</i> (3 high-yielding/hybrids + 3 low-yielding/hybrids)	intra-genotypic competition	ne ne ear leaves	F	UP: amino acid degradation/polyamine metabolism, cell wall degradation, glycolysis cytosolic branch, hormone metabolism (auxin), RNA transcription regulation, signaling receptor kinases and tetrapyrrole synthesis DOWN: flavonoids secondary metabolism, miscellaneous enzyme families, protein folding, protein post-translational modification and development	Choe et al., 2016
	heterospecific	<i>Arabidopsis thaliana</i>	<i>Heracleum pilosella</i>	ne ne roots	C	UP: photosynthesis, biotic stress response, transcription factors, signaling lipids DOWN: tricarboxylic acid, amino acid metabolism, glucosinolate	Schmidt et al., 2013
		<i>Centaurea maculosa</i>	<i>Gaillardia aristata</i> , <i>Festuca idahoensis</i>	ne ne leaves and roots	C	UP (in presence of each species): ADP ribosylation DOWN (in presence of each species): ABC transporter	Brozet et al., 2008
		<i>Glycine max</i>	<i>Abutilon theophrasti</i> , <i>Helianthus annuus</i> , <i>Linum usitatissimum</i> , <i>Polygonum convolvulus</i> , <i>Brassica napus</i>	ne ne leaves	F+C	UP: phytochrome signaling, photosynthesis, shade avoidance, oxidative stress responses, salicylic acid DOWN: heat shock response, protein synthesis, jasmonate acid signalling	Horvath et al., 2015
		<i>Solanum nigum</i>	<i>Plantago lanceolata</i> , <i>Lolium perenne</i> , <i>Trifolium pratense</i>	ne ne leaves	F+C	UP (in presence of each species): signal response, defense response	Schmidt and Baldwin, 2006
		<i>Trifolium lucidum</i>	<i>Trifolium maculab</i>	ne ne roots	C	DOWN: photosynthesis UP: abiotic and biotic stress, ethylene responses, protein synthesis and photosynthesis, disease resistance, heat shock response DOWN: disease resistance	Bowsher et al., 2017
		<i>Zea mays</i>	<i>Abutilon theophrasti</i>	ne ne leaves	F	DOWN: carbon and nitrogen utilization, photosynthesis, oxidative stress, signal transduction, responses to auxin and ethylene, zinc transport	Horvath et al., 2006
		<i>Zea mays</i>	<i>Abutilon theophrasti</i> , <i>Brassica napus</i>	ne ne leaves	F	UP: 13-LOX, 13-HPL, nutrient reservoir activity, meristem identity activity, protein kinase activity DOWN: light/photosynthesis, energy conversion, transcription	Moriles et al., 2012

**Figure 2.** Studies on global change in gene expression and QTL mapping studies reporting the genetics of natural plant-plant interactions. <sup>a</sup>GWA, Genome-Wide Association; QTL mapping, quantitative trait loci; <sup>b</sup>BILs, backcross inbred lines; RILs, recombinant inbred lines; SILs, segmental introgressions lines; <sup>c</sup>CE, competitive effect; CR, competitive response, ne, not estimated; <sup>d</sup>Numbers in brackets indicate the number of measured above-ground/below-ground traits. One number stands for the number of traits measured either for competitive response or for competitive effect, whereas two numbers stand for the number of traits measured for competitive response and competitive effect. The measured traits are listed in Data Set S1; <sup>e</sup>Growth conditions (greenhouse and growth chambers); F, field conditions; IS, *in situ*; <sup>f</sup>Genetic architecture; M, monogenic; P, polygenic; ne, not estimated; <sup>g</sup>UP and DOWN correspond to an up-regulation and down-regulation of genes associated with a specific functional category. nr, not reported. Candidate genes highlighted in red correspond to functionally validated genes.

Approach <sup>a</sup>	Type of interaction	Focal species <sup>b</sup>	Neighbor-species/Treatment	CR <sup>c</sup>	CE <sup>c</sup>	Plant organ <sup>d</sup>	Growth cond. <sup>e</sup>	Gen. Arch. <sup>f</sup>	Gen. Arch. <sup>f</sup>	Candidate genes <sup>g</sup>	Reference <sup>h</sup>
Global change in gene expression	asymmetric parasitism										
	heterospecific	<i>Medicago truncatula</i>	<i>Oronchche crenata</i>	ne	ne	roots	C	ne	ne	UP: cell wall degradation and modification, primary metabolism, defense response DOWN: primary metabolism, defense response and cell rescue	Dita <i>et al.</i> , 2009
		<i>Oryza sativa</i> (resistant cultivar)	<i>Striga hermonthica</i>	ne	ne	roots	C	ne	ne	UP: defense response, ABC transporters, phenylpropanoid metabolism, WRKY transcription factors DOWN: nutrient transporters, enzymes of amino acid metabolism	Swarbrick <i>et al.</i> , 2008
		<i>Oryza sativa</i> (susceptible cultivar)	<i>Striga hermonthica</i>	ne	ne	roots	C	ne	ne	UP: nutrient transporters, enzymes of amino acid metabolism DOWN: plant growth regulator signaling and metabolism, biogenesis of cellular components and cell division	Swarbrick <i>et al.</i> , 2008
		<i>Oryza sativa</i>	<i>Striga hermonthica</i>	ne	ne	roots	C	ne	ne	UP: defense response, jasmonic acid, salicylic acid <b>WRKY5</b>	Mutuku <i>et al.</i> , 2015
		<i>Vigna unguiculata</i>	<i>Striga gesnerioides</i> - incompatible interaction	ne	ne	roots	C	ne	ne	UP: signal transduction, programmed cell death and apoptosis, components of lignification, secondary wall formation DOWN: cell cycle regulators, cellular transporters	Huang <i>et al.</i> , 2012
		<i>Vigna unguiculata</i>	<i>Striga gesnerioides</i> - compatible interaction	ne	ne	roots	C	ne	ne	UP: cellular transport processes for nitrogen and sulfur DOWN: defense pathways, lignin biosynthesis, secondary wall modifications, plant growth regulators	Huang <i>et al.</i> , 2012
QTL mapping	competition conspecific										
Traditional QTL mapping	competition conspecific	<i>Arabidopsis thaliana</i> (411 RILs)	only presence of inter-genotypic competition	X	X	above-ground (n=9 +9)	C	P	P	<i>ACS4</i> , <i>ACS10</i> , <i>ASA1</i>	Mutt and Wolf, 2007
		<i>Arabidopsis thaliana</i> (three RIL populations, 340 RILs)	absence and presence of inter-genotypic competition	X		above-ground (n=2)	C	P	P	<i>PHYB</i> , <i>FIC</i> , <i>FR1</i>	Botto and Coluccio, 2007
		<i>Oryza sativa</i> × <i>Oryza rufipogon</i> (95 F <sub>2</sub> RILs)	intra-genotypic competition (two density conditions)	X	X	above-ground (n=2 +1)	F	P	nr		Shimizu and Itoh, 2012
		<i>Oryza sativa</i> × <i>Oryza rufipogon</i> (89 F <sub>2</sub> RILs)	intra-genotypic competition (three density conditions)	X		above-ground (n=4)	C	P	nr		Shimizu <i>et al.</i> , 2010
		<i>Zea mays</i> (8 SILs)	intra-genotypic competition (two density conditions)	X		above-ground (n=12)	F	P	nr		Gonzalo <i>et al.</i> , 2006
		<i>Zea mays</i> (186 RILs)	intra-genotypic competition (two density conditions)	X		above-ground (n=8)	F	P	nr		Gonzalo <i>et al.</i> , 2010
		<i>Zea mays</i> (231 F <sub>2.3</sub> families)	intra-genotypic competition (two density conditions)	X		above-ground (n=12)	F	P	nr		Guo <i>et al.</i> , 2011
		<i>Zea mays</i> (four RIL families, 840 RILs)	intra-genotypic competition (two density conditions)	X		above-ground (n=3)	F	P	nr		Ku <i>et al.</i> , 2015
		<i>Zea mays</i> (four RIL families, 858 RILs)	intra-genotypic competition (three density conditions)	X		above-ground (n=6)	F	P	nr		Ku <i>et al.</i> , 2016
		<i>Zea mays</i> (220 F <sub>30</sub> RILs)	intra-genotypic competition (two density conditions)	X		above-ground (n=3)	F	P	nr		Wang <i>et al.</i> , 2017
	heterospecific	<i>Oryza sativa</i> × <i>Oryza rufipogon</i> (274 BC <sub>2</sub> families)	<i>Brachiaria brizantha</i>	X		above-ground (n=7)	F	P	nr		Moncada <i>et al.</i> , 2001
		<i>Triticum aestivum</i> (161 Doubled Haploid lines)	<i>Lolium rigidum</i>	X	X	above-ground (n=9 +1)	F	P	nr		Coleman <i>et al.</i> , 2001
		<i>Triticum aestivum</i> (163 F <sub>2</sub> -derived RILs)	multiple weed species (conventional vs organic plots)	X	X	above-ground (n=9 +1)	F	P	nr	<i>RHT-81</i>	Asif <i>et al.</i> , 2015
		<i>Triticum aestivum</i> (140 F <sub>2</sub> -derived RILs)	<i>Lolium perenne</i>	X	X	above-ground (n=6 +1)	F	P	P	<i>Ppd1-B1</i> , <i>Vrn-B1</i> , <i>Rht</i> , <i>Vrn3</i>	Granberry, 2016

Figure 2. Continued

Approach <sup>a</sup>	Type of interaction	Focal species <sup>b</sup>	Neighbor species/Treatment	CR <sup>c</sup>	CE <sup>d</sup>	Plant organ <sup>d</sup>	Growth cond. <sup>e</sup>	Gen. Arch. <sup>f</sup>	Candidate genes <sup>g</sup>	References <sup>h</sup>
QTL mapping Traditional QTL mapping	asymmetric allelopathy heterospecific	<i>Oryza sativa</i> (121 RILs)	<i>Echinochloa crus-galli</i>	X		below-ground (n=1)	C	P	nr	Jensen et al., 2001
		<i>Oryza sativa</i> (192 F <sub>2</sub> families)	<i>Lactuca sativa</i>	X		below-ground (n=1)	C	P	nr	Bana et al., 2001 Okuno and Ebana, 2003
		<i>Oryza sativa</i> (F <sub>23</sub> families)	<i>Echinochloa crus-galli</i>	X		above-ground (n=1)	C	P	nr	Lee et al., 2005
		<i>Oryza sativa</i> (123 Doubled Haploid lines)	<i>Lactuca sativa</i>	X		below-ground (n=1)	C	P	nr	Zeng et al., 2003
		<i>Oryza sativa</i> (150 RILs)	<i>Echinochloa crus-galli</i>	X		above-ground (n=2) +below-ground (n=2)	C	P	nr	Jensen et al., 2008
		<i>Triticum aestivum</i> (271 Doubled Haploid lines)	<i>Lolium rigidum</i>	X		below-ground (n=1)	C	P	nr	Wu et al., 2003
		<i>Triticum aestivum</i> (277 F <sub>23</sub> families)	weeds + <i>Zea mays</i> + <i>Lolium rigidum</i>	X		above-ground (n=1+4) +below-ground (n=0+1)	F	P	nr	Zuo et al., 2012
		<i>Helianthus annuus</i> (230 F <sub>2</sub> families + 204 F <sub>2</sub> families)	<i>Orobanchae cumana</i>	X		above-ground (n=1)	C	M	nr	Lu et al., 2000
		<i>Helianthus annuus</i> (113 F <sub>2</sub> families)	<i>Orobanchae cumana</i>	X		above-ground (n=1) +below-ground (n=1)	F	P	nr	Pérez-Vich et al., 2002
		<i>Helianthus annuus</i> (262 RILs)	<i>Orobanchae cumana</i>	X		above-ground (n=1) +below-ground (n=1)	C	M	nr	Tang et al., 2003
parasitism heterospecific	<i>Helianthus annuus</i> (101 RILs)	<i>Orobanchae cumana</i>	X		above-ground (n=1) +below-ground (n=2)	F+C	P	nr	Louarn et al., 2015	
	<i>Helianthus annuus</i> (96 F <sub>23</sub> families)	<i>Orobanchae cumana</i>	X		below-ground (n=1)	C	M	nr	Imerovski et al., 2016	
	<i>Oryza sativa</i> (98 BILs)	<i>Striga hermonthica</i>	X		below-ground (n=1)	C	P	nr	Gurney et al., 2006	
	<i>Oryza sativa</i> (141 BILs)	<i>Striga hermonthica</i>	X		below-ground (n=1)	C	P	nr	Swarbrick et al., 2009	
	<i>Oryza sativa</i> (115 F <sub>6</sub> RILs)	<i>Striga hermonthica</i>	X		above-ground (n=6+2)	C	P	nr	Kaewchumnaong and Pries, 2008	
	<i>Oryza sativa</i> (115 F <sub>6</sub> RILs)	<i>Striga hermonthica</i>	X		above-ground (n=2+0) +below-ground (n=2+1)	C	P	SIB1 & SB12 (two cytochrome P450 genes)	Cardoso et al., 2014	
	<i>Pisum sativum</i> (115 RILs)	<i>Orobanchae crenata</i>	X		above-ground (n=1)	F	P	nr	Valderrama et al., 2004	
	<i>Pisum sativum</i> (111 F <sub>23</sub> RILs)	<i>Orobanchae crenata</i>	X		above-ground (n=0+1) +below-ground (n=1+3)	F+C	P	nr	Fondevilla et al., 2010	
	<i>Sorghum bicolor</i> (226 RIPS + 226 RIPS)	<i>Striga hermonthica</i>	X		above-ground (n=1)	F	P	nr	Hausmann et al., 2004	
	<i>Sorghum bicolor</i> (354 RILs)	<i>Striga asiatica</i> + <i>Striga hermonthica</i>	X		below-ground (n=1)	C	M	nr	Satish et al., 2012	
	<i>Sorghum bicolor</i> (selected RILs from 354 RILs)	<i>Striga asiatica</i> + <i>Striga hermonthica</i>	X		below-ground (n=1+1)	C	M	LG51 (sulfatransferase)	Gobena et al., 2017	

Figure 2. Continued

Approach <sup>a</sup>	Type of interaction	Focal species <sup>b</sup>	Neighbor species/Treatment	CR <sup>c</sup> CE <sup>c</sup> Plant organ <sup>d</sup>	Growth cond. <sup>e</sup>	Gen. Arch. <sup>f</sup>	Candidate genes <sup>g</sup>	References <sup>h</sup>
QTL mapping Traditional QTL mapping	asymmetric parasitism heterospecific	<i>Vicia faba</i> (196 F <sub>2</sub> families)	<i>Orobancha crenata</i>	X above-ground (n=1)	F	P	nr	Roman <i>et al.</i> , 2002
		<i>Vicia faba</i> (144 RILs)	<i>Orobancha betida</i>	X above-ground (n=1)	F	P	nr	Diaz-Ruiz <i>et al.</i> , 2009
		<i>Vicia faba</i> (156 F <sub>2</sub> RILs)	<i>Orobancha crenata</i>	X above-ground (n=1)	F	P	nr	Diaz-Ruiz <i>et al.</i> , 2010
		<i>Vicia faba</i> (119 F <sub>2</sub> -F <sub>8</sub> RILs)	<i>Orobancha crenata</i>	X above-ground (n=1)	F	P	nr	Gutiérrez <i>et al.</i> , 2013
		<i>Vicia faba</i> (119 F <sub>2</sub> -F <sub>8</sub> RILs)	<i>Orobancha betida</i>	X above-ground (n=1)	F	P	nr	Gutiérrez <i>et al.</i> , 2013
		<i>Vigna unguiculata</i> (four 150 F <sub>2</sub> families + 153 F <sub>3</sub> lines)	<i>Striga gesnerioides</i>	X above-ground (n=1)	F	M	nr	Ouédrago <i>et al.</i> , 2001
		<i>Vigna unguiculata</i> (two 150 F <sub>2</sub> families)	<i>Striga gesnerioides</i>	+ below-ground (n=1)	F	P	nr	Ouédrago <i>et al.</i> , 2002
		<i>Vigna unguiculata</i> (150 F <sub>2</sub> RILs)	<i>Striga gesnerioides</i>	X above-ground (n=1)	C	M	R93-301 (CC-NBS-LRR)	Li and Timko, 2009
		<i>Vigna unguiculata</i> (62 F <sub>2</sub> families + 35 F <sub>2</sub> families)	<i>Striga gesnerioides</i>	X below-ground (n=1)	C	M	nr	Boular <i>et al.</i> , 2004
		GWA mapping	competition conspecific	<i>Arabidopsis thaliana</i> (48 local accessions)	absence and presence of intra-genotypic competition	X X above-ground (n=9)	F	P
<i>Oryza sativa</i> (801 accessions + 151 MAGIC lines)	intra-genotypic competition (two density conditions)			X above-ground (n=9)	F	P	O403g0275400	Kikuchi <i>et al.</i> , 2017
<i>Arabidopsis thaliana</i> (48 local accessions)	<i>Poa annua</i> , <i>Stelaria media</i> , <i>Trifolium repens</i> , <i>Veronica arvensis</i>			X X above-ground (n=8 +1)	F	P	HAM2, AT5G26670, AT5G66530	Baron <i>et al.</i> , 2015
<i>Arabidopsis thaliana</i> (195 local accessions)	<i>Poa annua</i>			X above-ground (n=29)	IS	P	FLC, T5F	Frachon <i>et al.</i> , 2017

Figure 2. Continued

interactions have been subsequently tested for a role in direct interactions with neighboring plants (e.g. Schmitt *et al.*, 1995; Bates and Lynch, 2001; Cipollini, 2002; Fitter *et al.*, 2002; Cahill *et al.*, 2005), no studies have reported a direct EMS mutant screen in the presence of conspecifics or heterospecifics (Figure 2). This discrepancy in EMS mutant screens between plant–plant interactions and other types of biotic interactions may originate from the complexity of the establishment of the experiment involving interactions with neighbor plants. While screening for EMS mutants impaired in their interactions with microbial partners often requires only the spraying of a microbial solution on tens of thousands of seedlings sown at a high density, screening EMS mutants involved in plant–plant interactions would require the individual planting of the same number of seeds in presence of a neighbor plant.

Secondly, 16 of the 63 studies correspond to analysis of global change in gene expression (Figure 2). Among the remaining studies, 44 studies are based on traditional QTL mapping approaches using diverse experimental populations ( $F_2$  populations, recombinant inbred lines, Doubled Haploid lines or back-crossed lines), while three studies correspond to GWAS that have been all published in the last 3 years (Figure 2). These GWAS are directly linked to the recent development of the next generation sequencing (NGS) technologies (Goodwin *et al.*, 2016; Lee *et al.*, 2016) that provide a substantial number of diverse genetic markers covering the whole genome [i.e. single nucleotide polymorphisms (SNPs); copy number variation (CNV); indels, insertion–deletion], thereby allowing the fine mapping of genes underlying natural variation of complex traits (Bergelson and Roux, 2010). Although most GWAS in plants have been based on genetic lines collected over the entire geographic area of the studied plant species (Bartoli and Roux, 2017), we must however remember that a mapping population should be chosen according to the spatial scale at which natural variation is observed, i.e. according to the spatial scale of the ecological factors acting as selective pressures on the studied trait (Bergelson and Roux, 2010). For example, the significance level of association peaks for phenological variation was higher at the regional scale than at the worldwide scale in *A. thaliana* (Brachi *et al.*, 2013). Because plants interact with neighbors over short distances, the use of highly genetically polymorphic local populations to fine map QTLs underlying plant–plant interactions appears to be more suitable than using worldwide genetic lines (Baron *et al.*, 2015). Therefore, in order to accelerate the identification of QTLs underlying natural variation of plant–plant interactions, we advocate the development of local mapping populations that are known to interact with other plant species and are genetically diverse. While such populations can be identified within wild species (Frachon *et al.*, 2017), this may remain an important challenge in major crops.

Thirdly, the 16 transcriptomic studies are unevenly distributed between competitive interactions ( $n = 12$  studies, equally distributed between interactions with conspecifics and interactions with heterospecifics) and asymmetric interactions ( $n = 4$  studies, all focusing on response to parasitic plants) (Figure 2). An opposite pattern is observed for QTL mapping studies. The 47 QTL mapping studies are unevenly distributed between competitive interactions (approximately one-third), mostly testing intra-genotypic interactions (i.e. density effect), and asymmetric interactions (approximately two-thirds) (Figure 2). In the latter case, all the 30 corresponding studies were based on heterospecific interactions in the context of either allelopathy underlying weed suppressive ability ( $n = 8$  studies) or response to parasitic plants ( $n = 22$  studies) (Figure 2). Surprisingly, no studies have been carried out on the identification of genetic and molecular bases underlying natural variation of positive interactions, such as facilitation and mutualism at the interspecific level and cooperation at the intraspecific level. It is a fact that negative plant–plant interactions, in particular competitive interactions, are thought of as the major factor responsible for crop yield reduction and for determining the structure of natural plant communities. However, this view has been recently challenged by several studies and the role of positive interactions (mostly facilitation) on overyielding in crop mixtures and in regulating the composition of natural plant communities has gained a lot of attention (Bertness and Callaway, 1994; Callaway, 1995; Brooker and Callaghan, 1998; Bruno *et al.*, 2003; Brooker *et al.*, 2008; Bukowski and Petermann, 2014; Li *et al.*, 2014; Wendling *et al.*, 2017). The next challenge is therefore the identification of candidate genes underlying positive interactions in various plant–plant interacting systems, which would enable testing whether some signaling pathways involved in response to neighbor presence are shared between competitive, asymmetric and reciprocal helping interactions.

Fourthly, 51 of the 63 studies involved 10 crop species as focal plants, distributed across three botanical families, i.e. Asteraceae (*Helianthus annuus*), Fabaceae (*Glycine max*, *Pisum sativum*, *Vicia faba* and *Vigna unguiculata*) and Poaceae (*Hordeum vulgare*, *Oryza sativa*, *Sorghum bicolor*, *Triticum aestivum* and *Zea mays*) (Figure 2). This major interest in crop species is consistent with the economic and environmental cost of crop weeds (Neve *et al.*, 2009) and with breeding programs for more density-related tolerant cultivars (St. Pierre *et al.*, 2011). Interestingly, while some crop species have been mainly studied for a specific type of plant–plant interactions such as *Zea mays* for response to intra-genotype competition, other crop species have been studied for diverse types of plant–plant interactions such as *Oryza sativa* for competitive interactions with conspecifics and heterospecifics, for allelopathic effects on weeds and for response to parasitic plants

(Figure 2). The remaining focal species correspond to five wild species, i.e. *A. thaliana* ( $n = 8$  studies), *Centaurea maculosa* ( $n = 1$ ), *Medicago truncatula* ( $n = 1$ ), *Solanum nigrum* ( $n = 1$ ) and *Trifolium fucatum* ( $n = 1$ ) (Figure 2). In comparison with other types of biotic interactions (Roux and Bergelson, 2016; Bartoli and Roux, 2017), we identified only four QTL studies of plant–plant interactions in *A. thaliana*. This paucity of studies may stem from its status as a pioneer species; i.e. *A. thaliana* is not considered as being often challenged by other plant species in its natural habitats. However, several studies recently challenged this view: (i) by revealing extensive genetic diversity in *A. thaliana* for the response to intra- and interspecific competition (Bartelheimer *et al.*, 2015); (ii) by finding that plant–plant interactions may act as selective agents on phenology in *A. thaliana* (Brachi *et al.*, 2012, 2013); and (iii) by demonstrating the *in situ* adaptive evolution of a highly genetically polymorphic local population of *A. thaliana* to increased interspecific competition in less than eight generations (Frachon *et al.*, 2017). Therefore, *A. thaliana* appears as a valuable model system for studying the genetics of natural variation of plant–plant interactions. On the side of the neighbor species, 35 species have been used to study the genetics of plant–plant interactions (Figure 2). This number, which is 2.3 times higher than that of the focal species, well illustrates the diversity of plant species faced by crop species in fields and wild species in natural settings (Wilson *et al.*, 2012). To summarize, we identified 38 plant–plant interacting systems, corresponding to five conspecific and 33 heterospecific interacting systems (Figure 2). Obviously, these interacting systems represent only a tiny fraction of the interactions shared between a species and its neighbors, particularly in natural environments. There is therefore an urgent need to increase the diversity of the plant–plant interacting systems studied, in particular for wild species. Such a diversity would certainly help to obtain a broader view of the pathways involved in the detection and response to the presence of neighbors.

Fifthly, the ability of a focal plant to interact with its neighbor plants results from both its competitive response (i.e. how strongly the focal plant is affected by its neighbors) and its competitive effect (i.e. how strongly a focal plant affects the performance of its neighbors) (Bartelheimer *et al.*, 2015). However, 36 of the 47 QTL studies reported the identification of QTLs of either one or the other component (Figure 2). In addition, among these QTL studies, the component of competitive ability under study was highly specific to the type of plant–plant interactions. Most studies on competitive interactions and asymmetric interactions reported the identification of QTLs associated with competitive response and competitive effect, respectively (Figure 2). Interestingly, in studies reporting identification of QTLs for both components ( $n = 11$  studies), QTLs

of competitive response barely overlap with QTLs of competitive effect. While this observation suggests that competitive response may evolve independently from competitive effect (Baron *et al.*, 2015), we stress that considering simultaneously both competitive response and competitive effect would undoubtedly help to obtain an unbiased picture of the genetic and molecular mechanisms underlying the ability of a focal species to interact with its neighbor plants.

Sixthly, for competitive interactions, both above-ground (leaves) and below-ground (roots) traits have been used to study the global change of gene expression of a focal species in presence of a neighbor species, whereas all the traits measured in QTL mapping studies are exclusively above-ground (Figure 2). An opposite pattern was observed for asymmetric interactions. Roots have been exclusively used in transcriptomic studies on the response to parasitic plants, while both above-ground ( $n = 36$ ) and below-ground ( $n = 27$ ) traits have been measured in QTL mapping studies (Figure 2). We also observed a higher number of measured traits in QTL studies on competitive interactions (mean = 8.9 traits / study, median = 8.5 traits / study) than in QTL studies on asymmetric interactions (mean = 2.1 traits / study, median = 1 trait / study) (Figure 2). These observations are consistent with the difficulty of having access to the root compartment, especially in the case of QTL mapping studies that typically involve phenotyping several hundred or even thousands of individuals. The next frontier is therefore the development of high-throughput phenotyping for the precise root–root interactions (Mommer *et al.*, 2016). This challenge is already starting to be achieved by the development of image-analysis tools enabling quantitative analysis of root system architecture (Lobet *et al.*, 2011, 2013). For example, the use of a transparent gel system combined with image analysis and 3D reconstruction has allowed sophisticated analysis of the response of rice roots to another plant or physical object (Fang *et al.*, 2013). This study revealed a coordinated root system response integrating rhizosphere signals into root architecture showing genotype-specific root recognition via root tip signaling. However, experiments in controlled conditions can lead to artificial plant responses and results need to be confirmed in less artificial conditions. Recently, novel methods have been developed to document the 3D root system architecture within natural or field soils, using non-invasive (ground-penetrating radar for trees; Isaac and Anglaere, 2013) or low-invasive tools (minirhizotrons; Johnson *et al.*, 2001). In addition, below-ground DNA-based techniques have been recently developed for quantifying species proportions in mixed root samples (Mommer *et al.*, 2008, 2011), thereby allowing the study of below-ground species richness and rooting distributions (Jones *et al.*, 2011; Kesanakurti *et al.*, 2011) that can ultimately be linked to above-ground

abundance (Frank *et al.*, 2010). Nevertheless, non-invasive access to below-ground plant traits remains a daunting challenge, especially when studying multispecies mixtures.

Seventhly, the environmental conditions in which phenotyping of plant–plant interactions was performed are well balanced between controlled (greenhouse/growth chamber) conditions ( $n = 32$  studies) and field conditions ( $n = 26$ ) (Figure 2). As expected, the majority of studies on global change in gene expression were performed in controlled conditions in order to reduce variation among biological replicates. Conversely, one transcriptomic study has taken this habit out of step, by challenging soybean plants with different weed species over 3 years successively under field conditions (Horvath *et al.*, 2015). This procedure allowed the authors to detect genes with consistent differential expression over the three growing seasons, thus uncovering genes underlying general soybean responses to weed presence. Four of the five remaining studies reported phenotyping experiments in both controlled and field conditions (Schmidt and Baldwin, 2006; Fondevilla *et al.*, 2010; Horvath *et al.*, 2015; Louarn *et al.*, 2016). While controlled and field conditions are complementary, natural selection acts in nature, where the neighbor plants and associated cues are numerous and complex. We therefore argue that identifying genes that underlie natural variation of plant–plant interactions under natural conditions will be crucial for understanding the adaptation to the presence of neighbors, especially in wild species. Accordingly, a recent study reported for the first time (to our knowledge) a GWA mapping approach combined with an *in situ* phenotyping experiment of heterospecific interactions (Frachon *et al.*, 2017). In this study, 195 whole-genome sequenced natural accessions collected in a highly genetically polymorphic local population of *A. thaliana* were phenotyped *in situ* for 29 above-ground traits in six representative micro-habitats, consisting of the presence or absence of the bluegrass *Poa annua* (a species frequently associated with *A. thaliana* in its natural communities) crossed with three contrasting soil types. Interestingly, a minor fraction of the SNPs the most highly associated with the response to the presence of *P. annua* was shared among the three soil types, stressing the need to consider the impact of abiotic conditions on the identification of the genetic bases underlying competitive ability in a heterospecific neighborhood (Frachon *et al.*, 2017). Further experiments conducted under natural conditions will undoubtedly help to unravel the complexity of the molecular and genetic bases underlying natural plant–plant interactions.

Finally, in agreement with other types of biotic interactions (Bartoli and Roux, 2017), the majority of QTL mapping studies ( $n = 39$ ) revealed a complex genetic architecture associated with plant–plant interactions (Figure 2).

The quantitative genetic architecture is highly diverse among plant–plant interacting systems, ranging from the identification of few medium-effect QTLs to the identification of up to tens of small-effect QTLs (Frachon *et al.*, 2017). A monogenic architecture was reported for the remaining eight QTL mapping studies, all focusing on the natural variation of response to parasitic plants in three crop species, i.e. *H. annuus* ( $n = 3$  studies), *S. bicolor* ( $n = 2$  studies) and *V. unguiculata* ( $n = 3$  studies). While there is a temptation to focus on cloning QTLs underlying binary traits, a polygenic architecture is more in line with theoretical works on adaptive walk to phenotypic optima (Hermisson and Pennings, 2005; Orr, 2005). Cloning medium (<30%) and small (<10%) effect QTLs rather than large-effect QTLs may therefore reveal genes involved in the adaptive response to the presence of a neighbor. Nonetheless, we should keep in mind that the functional validation of QTLs explaining less than 10% of phenotypic variation can require the phenotyping of up to thousands of plants, thereby explaining the scarcity of studies reporting the cloning of genes underlying small-effect QTLs whatever the type of biotic interactions considered (Bergelson and Roux, 2010; Roux and Bergelson, 2016).

#### The genetic and molecular bases underlying natural plant–plant interactions

It comes as no surprise that many more candidate genes were identified in the studies on global changes in gene expression than in the QTL mapping studies (in particular the traditional QTL mapping studies) (Figure 2). Although the identity of the candidate genes barely overlaps between transcriptomics studies and QTL mapping studies, the functions of candidate genes are very overlapping (Figure 2). The candidate genes can be classified into seven categories of plant function that have been identified in studies based on artificial environments designed to simulate plant–plant interactions either frequently (photosynthesis and hormones), only recently (cell wall modification and degradation, defense pathways against pathogens) or rarely (ATP-binding cassette ABC transporters, histone modification, meristem identity/life history traits). This observation highlights the complementarity of identifying the genetic and molecular mechanisms underlying plant–plant interactions in artificial environments simulating plant–plant interactions and in environments where focal plants have been directly challenged by neighbor plants. We should also mention that: (i) very few candidate genes have been identified as being involved in nutrient competition (Masclaux *et al.*, 2012); (ii) the function of the up-regulated and down-regulated genes can be highly dependent on the genotype tested, as found in barley and maize (St. Pierre *et al.*, 2011; Choe *et al.*, 2016); and (iii) several studies reported a substantial fraction of genes with unknown functions in their list of

candidate genes (Horvath *et al.*, 2006; Broz *et al.*, 2008; Swarbrick *et al.*, 2008; Dita *et al.*, 2009; Biedrzycki *et al.*, 2011a; Huang *et al.*, 2012; Schmid *et al.*, 2013; Baron *et al.*, 2015). The latter result suggests that some molecular mechanisms of neighbor perception and signaling pathways involved in the trigger of a response strategy remain to be identified.

Photosynthesis genes were specifically identified in presence of competitive interactions (Figure 2). In most cases, photosynthesis genes were up-regulated in presence of conspecifics or heterospecifics, likely in connection with the SAS. Accordingly, *PHYTOCHROME B* (*PHYB*) that plays a central role in determining plant responses to changes in the R:FR ration caused by the proximity of other plants was up-regulated both in barley and maize in high plant density conditions (St. Pierre *et al.*, 2011). In addition, in presence of inter-genotypic competition, *PHYB* was proposed as a candidate gene for an overlapping QTL among three RIL families of *A. thaliana*. Based on transgenic analysis, further study confirmed experimentally that natural *PHYB* polymorphisms in *A. thaliana* cause differential responses in light sensitivity (Filiault *et al.*, 2008). Three studies reported a down-regulation of photosynthesis genes, all in presence of heterospecifics (Horvath *et al.*, 2006; Schmidt and Baldwin, 2006; Moriles *et al.*, 2012). A putative explanation relies on the permanent inhibition of photosynthesis that is induced when the focal plant is challenged in its early development by a neighbor plant, even if the focal plant overtops the neighbor plant later during its life cycle (Horvath *et al.*, 2006; Moriles *et al.*, 2012). The relative growth stage between two competing plants may therefore condition regulation of their photosystem genes.

The signal transduction network involved in SAS targets major physiological regulatory components such as the growth-associated hormones auxin, ethylene and gibberellins, whose biosynthesis is stimulated upon exposure to low R:FR ratios (Ballaré and Pierik, 2017). In agreement with the expression changes observed in photosystem genes, hormone-related genes were specifically detected in presence of competitive interactions (Figure 2). In competition with conspecifics, auxin-related genes were up-regulated in barley and maize (St. Pierre *et al.*, 2011; Choe *et al.*, 2016) and a subunit (*ASA1*) of anthranilate synthase, which is involved in auxin synthesis, was proposed as a candidate gene underlying a QTL of response to inter-genotypic competition in *A. thaliana* (Mutic and Wolf, 2007). Ethylene-related genes were up-regulated in *Trifolium fucatum* when competing with its congeneric *T. macraei* (Bowsher *et al.*, 2017). In addition, two candidates underlying two other QTLs of response to inter-genotypic competition in *A. thaliana* correspond to two polypeptides (*ACS4* and *ACS10*) involved in the formation of 1-amino-cyclopropane-1-carboxylate synthase (*ACS*), which governs the rate-limiting step in ethylene formation (Mutic and Wolf,

2007). A GWAS reported the fine mapping of a genomic region associated with the length of reproductive period in *A. thaliana* in response to the presence of *Veronica arvensis* (Baron *et al.*, 2015). This genomic region of 30 kb contains the gene *AT5G66350* that codes for the SHI (for Short Internodes) protein involved in the perception of or in the response to gibberellin (Fridborg *et al.*, 1999).

Cell wall modification and degradation are important components of cell expansion, which is the driving force of organ elongation (Ballaré and Pierik, 2017). In line with the cell growth machinery being the ultimate target of the signal transduction network involved in SAS and hormone-related pathways, genes related to cell wall modification and degradation were up-regulated in presence of intraspecific competition (Choe *et al.*, 2016; Bowsher *et al.*, 2017). A genomic region of less than 10 kb associated with the length of reproductive period in *A. thaliana* has been fine mapped in response to the presence of *Stellaria media* (Baron *et al.*, 2015). The underlying candidate gene corresponds to the pectin acetyltransferase gene *AT5G26670*, which encodes a cell wall modification protein regulated by VOCs emitted by the rhizobacterium *Bacillus subtilis* (Zhang *et al.*, 2007). The latter case suggests that microbial-mediated below-ground communications between two plant species can ultimately lead to an above-ground adaptive response strategy.

Expression changes of numerous genes associated with defense pathways against microbial pathogens and insects have been observed in different types of plant–plant interactions (Figure 2). Firstly, in response to parasitic plants, defense-related genes were up-regulated in incompatible interactions and down-regulated in compatible interactions. For example, the expression of *WRKY45*, a regulator of the salicylic acid/benzothiadiazole pathway, was highly induced in *Striga hermonthica*-infected rice (Mutuku *et al.*, 2015). In a study on an incompatible interaction with *Striga gesnerioides*, an up-regulation was observed in *Vigna unguiculata* for genes underlying programmed cell death and apoptosis (Huang *et al.*, 2012). In addition, the authors noticed that some genes and pathways up-regulated in *V. unguiculata* during incompatible interactions were also repressed during compatible interactions, suggesting that specific components of the host defense can be targeted and/or manipulated by *S. gesnerioides*. In line with those observations, the cloning of the first resistance gene in *V. unguiculata* to *S. gesnerioides* led to the identification of a predicted coiled-coil nucleotide-binding site leucine-rich repeat (CC-NBS-LRR) resistance protein (*R* gene) (Li and Timko, 2009). While this result suggests that similar molecular functions are shared among interactions involving microbial pathogenicity and plant parasitism, two recent studies reported the identification and functional validation of three genes conferring resistance to *Striga* sp. and having molecular functions that are distinct from *R* genes. The

first study reported in rice was on the functional validation of two cytochrome P450 genes (*SBL1* and *SBL2* involved in the biosynthesis of the hormone strigolactone) as underlying a major QTL conferring resistance to the parasitic plant *S. hermonthica* (Cardoso *et al.*, 2014). The natural lines containing a deletion of *SBL1* and *SBL2* exuded lower amounts of strigolactone and had lower strigolactone content, thereby decreasing the rate of perception of the rice plants by *Striga* sp. (Cardoso *et al.*, 2014). The second study that was reported in sorghum contained the functional validation of *LGS1* (*LOW GERMINATION STIMULANT 1*) as underlying a major QTL conferring resistance to both *S. asiatica* and *S. hermonthica* (Gobena *et al.*, 2017). *LGS1* codes for an enzyme annotated as a sulfo-transferase. Independent functional losses of *LGS1* in sorghum cultivars result in changes of the type of strigolactones present in the root exudates, i.e. from the dominant strigolactone 5-deoxystrigol (a highly active *Striga* germination stimulant) to orobanchol, another strigolactone compound that does not stimulate *Striga* germination (Gobena *et al.*, 2017). Secondly, for competitive interactions, none of the candidate genes proposed in QTL mapping studies is related to defense pathways (Figure 2). In addition, no clear pattern of regulation in defense-related genes was observed among the transcriptomic studies. For example, for conspecific interactions in *A. thaliana*, two studies reported an up-regulation of defense-related genes (Biedrzycki *et al.*, 2011a; Masclaux *et al.*, 2012), while a third study reported the opposite pattern (Geisler *et al.*, 2012). Up-regulation and down-regulation of defense-related genes were even reported within the same studies (Horvath *et al.*, 2015; Bowsher *et al.*, 2017). The growth-defense balance theory predicts that light perception by photoreceptors activates SAS and reduces the expression of defenses against microbial pathogens and insects, by a simultaneous down-regulation of jasmonate and salicylic acid signaling (Ballaré, 2014; Ballaré and Pierik, 2017). While some transcriptomic studies support this trade-off (Schmidt and Baldwin, 2006; Geisler *et al.*, 2012), other studies suggest an independent regulation of the SAS-related pathways and defense pathways in the focal plant competing with a neighbor plant (Masclaux *et al.*, 2012; Horvath *et al.*, 2015; Bowsher *et al.*, 2017). Accordingly, some recent studies documented a reduction of disease severity in focal plants that were challenged by neighbor plants (Figure 3). For example, a reduction of symptoms caused on soybean by the pathogenic fungus *Cylindrocladium parasiticum* was achieved in controlled conditions by growing maize in the same pot (Gao *et al.*, 2014). The direct interaction of soybean with maize roots induced, in soybean roots, the expression of most *Pathogenesis-Related (PR)* genes tested as well as the *Phenylalanine Ammonia Lyase* gene (*PAL*; involved in the biosynthesis of secondary metabolites). Indeed, the use of

Plant combination <sup>1</sup>	Pathogen species	Symptom/damage fold reduction	Molecular immunity-related phenotype observed on focal plant in conspecific vs heterospecific <sup>3</sup>	References
Watermelon/Rice	<i>Fusarium oxysporum</i> f. sp. <i>niveum</i>	∞ <sup>2</sup>	several defense-related activities reduced (before infection)	Ren <i>et al.</i> , 2008
Soybean/Maize	<i>Cylindrocladium parasiticum</i>	1.7	<i>PR</i> , <i>PAL</i> and <i>PPO</i> gene induction enhanced (after infection)	Gao <i>et al.</i> , 2014
Watermelon/Wheat	<i>Fusarium oxysporum</i> f. sp. <i>niveum</i>	3.9	Induction of <i>AOS</i> , <i>PAL</i> and other genes enhanced (after infection but not before)	Xu <i>et al.</i> , 2015
Tomato/Onion	<i>Verticillium dahliae</i>	1.4	many genes involved in biotic response enhanced (RNASeq after infection)	Fu <i>et al.</i> , 2015

**Figure 3.** Evidence for modification of immunity by the plant neighborhood. <sup>1</sup>The first species is the focal plant on which measures (such as disease and immunity) were scored, whereas the second species corresponds to the identity of the neighbor species inducing changes; <sup>2</sup>0% in interspecific versus 66% in conspecific; <sup>3</sup>*AOS*, allene oxide synthase; *PAL*, phenyl ammonia lyase; *PPO*, polyphenol oxidase; *PR*, pathogenesis-related. n.t., not tested.

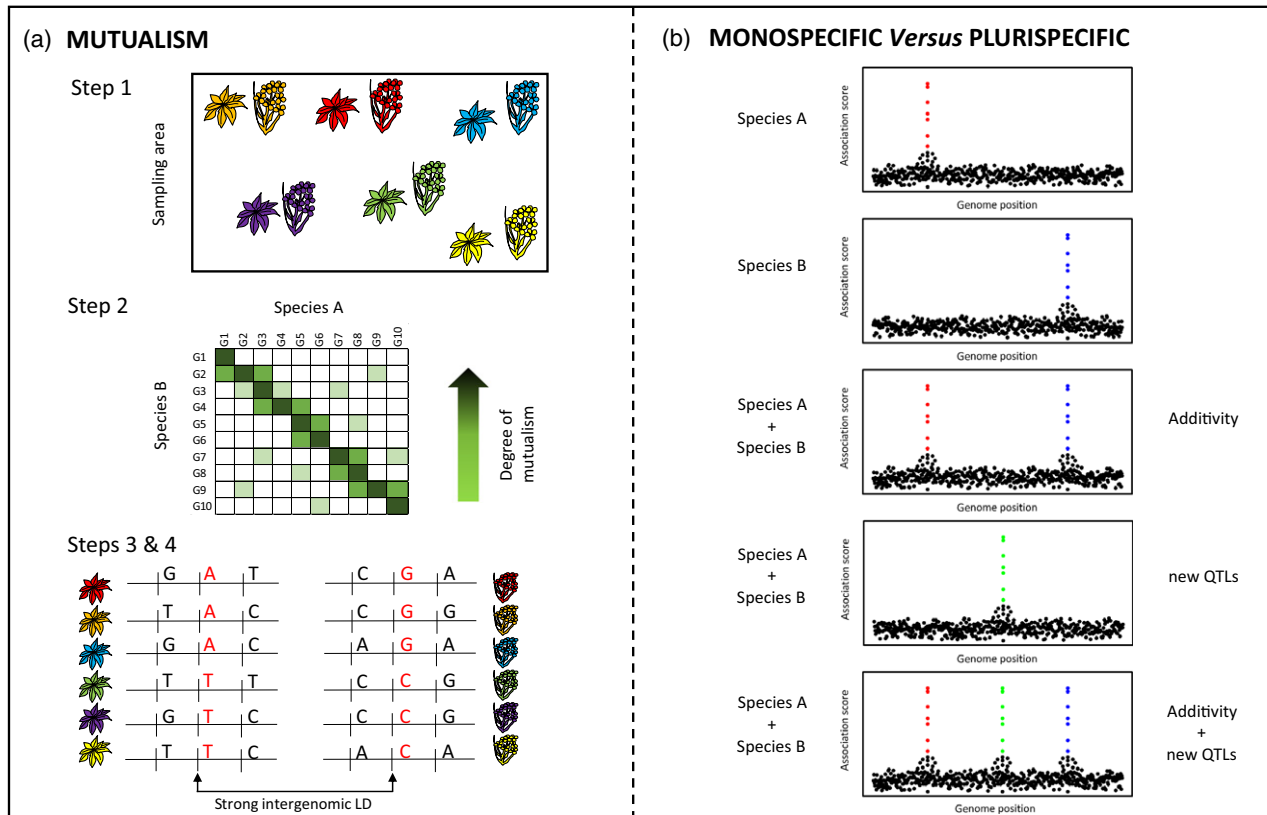
mesh or barrier separating the root systems from the two species showed that this induction of defense-related genes likely requires the diffusion of molecular signals from maize to soybean. Interestingly, exudates from maize were shown to contain salicylic acid, a potent inducer of SAR, which could also explain the induction of *PR* genes in soybean roots. Similar results were obtained in watermelon roots when grown together with wheat: *PAL* activity was higher in watermelon leaves and the induction of several defense-related genes was enhanced upon infection by pathogenic fungus *Fusarium oxysporum* (Xu *et al.*, 2015). In a couple of studies, exudates or purified molecules from root exudates produced by one plant species were shown to alter the expression of immunity markers in different plant species. For example, the expression of marker genes from several defense pathways was measured in shoots of healthy maize plants treated with root exudates from healthy pepper (Ding *et al.*, 2015). The induction of the allene oxide synthetase (AOS) and allene oxide cyclase (AOC), two genes involved in the biosynthesis of the jasmonic acid hormone, in maize roots, was further correlated with a reduction of lesions caused by the fungal necrotrophic pathogen *Bipolaris maydis* on maize leaves (Ding *et al.*, 2015). In addition, a slight accumulation in the leaves of the secondary metabolite DIMBOA, a naturally occurring hydroxamic acid, was observed. This molecule and its major derivatives were shown to have an antimicrobial activity on *B. maydis* *in vitro*, suggesting that exudates from pepper roots can trigger induced systemic resistance in maize (Ding *et al.*, 2015). More recently, it was shown that *p*-coumaric acid secreted by rice roots could induce *PR* gene expression in watermelon and protect it against *F. oxysporum* when directly applied to watermelon (Ren *et al.*, 2016), possibly explaining the disease reduction observed when the two species are grown together (Ren *et al.*, 2008). The discrepancy between studies supporting the growth-defense balance theory and studies reporting positive effects of competitive interactions on plant immunity may originate from the diversity of signals perceived by a focal plant. While the growth-defense balance theory is mainly based on the perception of a low R:FR ratio, the perception of other signals in a more realistic environment may modify the interconnections within the network of regulatory pathways involved in the response of a focal plant to neighbor plants. Further experimental studies are clearly needed to resolve this discrepancy.

The three following categories of plant functions have only rarely been highlighted in studies on plant–plant interactions. However, as these categories have been mentioned in several studies where focal plants were directly challenged by neighbor plants, they deserve a particular attention. Firstly, ABC transporter genes were up-regulated in two studies on conspecific interactions in *A. thaliana*

(Biedrzycki *et al.*, 2011a; Geisler *et al.*, 2012) and one study on response to the parasitic plant *S. hemonithica* in rice. In contrast, ABC transporter genes were down-regulated in one study on heterospecific interactions in *C. maculosa* (Broz *et al.*, 2008) (Figure 2). Originally identified as transporters involved in detoxification processes, ABC transporters have ever since been described for being involved in a large diversity of processes, such as transport of defense-related chemicals and phytohormones (Kang *et al.*, 2011; Kretschmar *et al.*, 2011; Hwang *et al.*, 2016). In the latter case, some ABC transporters are particularly essential to facilitate the communication between below- and above-ground structures, through the translocation of the signaling molecules cytokinins (Hwang *et al.*, 2016). An efficient communication system that coordinates the physiological and developmental processes between these two structures appears as a crucial point for a focal plant to quickly adopt an appropriate response strategy to a neighbor plant. Interestingly, the role of three ABC transporters in the kin recognition response was confirmed in *A. thaliana* (Biedrzycki *et al.*, 2011b). In particular, their expression levels increased in the roots of plants exposed to stranger root secretions versus those exposed to own or kin secretions. Further functional studies are needed to establish whether ABC transporters may also be involved in recognition of heterospecific strangers.

Secondly, a plethora of histone-related genes was shown to be down-regulated in barley plant at high density (St. Pierre *et al.*, 2011) (Figure 2). Based on the regulation of light-mediated chromatin compaction of the nuclear organizing regions (NORs) by *PHYB* and *HISTONE DEACETYLASE-6* in *A. thaliana* (Tessadori *et al.*, 2009), the authors proposed that the chromatin was more compact in plants grown at low density (i.e. with high light) than in plants grown at high density (i.e. with low light) (St. Pierre *et al.*, 2011). Furthermore, a GWAS reported the fine mapping of a genomic region associated with the number of basal branches in *A. thaliana* in response to the presence of *P. annua* (Baron *et al.*, 2015). This genomic region contains the gene *AT5G09740* that codes for the histone acetyltransferase HAM2 involved in the regulation of the expression of the well known pleiotropic gene *FLOWERING LOCUS C* (Xiao *et al.*, 2013), a MADS-box transcription factor that regulates branching patterns in *A. thaliana* (Huang *et al.*, 2013). Those observations involving histone-related genes are intriguing and deserve in-depth investigation to validate their putative roles in competitive interactions.

Thirdly, genes related to either floral meristem identity and/or life history traits (such as flowering time and seed dispersal linked to branching patterns) were specifically identified in competitive interactions (Figure 2). The identified candidate genes were either up-regulated in maize in heterospecific conditions (Moriles *et al.*, 2012) or proposed



**Figure 4.** Future avenues on the genetics underlying natural variation of plant–plant interactions. (a) Mutualism. Step 1: paired sampling of genotypes from species A and genotypes from species B. Step 2: Testing for mutualism based a small number of paired genotypes. Steps 3 and 4: whole-genome sequencing of both plants species and genome-to-genome statistical analysis. (b) Monospecific versus plurispecific heterospecific interactions. Hypothetical genetic architectures expected in a focal species in the context of plurispecific competition with two species A and B, as illustrated by Manhattan plots based on GWAS.

as underlying QTLs in five QTL mapping studies in both conspecific and heterospecific conditions (Botto and Coluccio, 2007; Asif *et al.*, 2015; Granberry, 2016; Frachon *et al.*, 2017; Kikuchi *et al.*, 2017) (Figure 2). In competitive environments, such candidate genes may trigger an adaptive escape strategy that would correspond to an increased reproductive efficiency, mediated by a shortening of the life cycle and a faster reallocation of vegetative resources to reproductive structures, which is itself facilitated by an increased number of branches (Bonser, 2013). In agreement with this hypothesis, in a natural plant community dominated by grasses, an adaptive evolution towards an escape strategy was observed in *A. thaliana* in less than eight generations (Frachon *et al.*, 2017). This response to increased interspecific competition was mediated in part by the meristem identity gene *TWIN SISTER OF FT* (*TSF*) found associated with bolting time, the length of reproductive period and the number of branches on the main stem (Frachon *et al.*, 2017). This result suggests that phenotyping life history traits can help to obtain a better understanding of the genetic and molecular mechanisms

underlying natural variation in plant–plant interactions, especially in wild plant species.

Although many studies proposed candidate genes involved in natural plant–plant interactions, only four of these studies have been followed up by studies aiming at functionally validating the causal genes (Figure 2). In QTL mapping studies, functional validation of candidate genes is however a pre-requisite to analyze the transcriptional and/or post-transcriptional regulation of the causal gene and to search for proteins directly interacting with the causal gene, that will in turn facilitate the identification of the downstream signaling pathways. Such complementary studies may then provide new candidate genes for breeding programs based on MAS.

**FUTURE AVENUES**

Here, we introduce several avenues that need to be explored in the future to obtain a thorough understanding of the genetic and molecular bases underlying plant–plant interactions within the context of realistic community complexity.

### Identifying the genetic and molecular bases underlying natural variation of mutualism

As previously mentioned, studies reporting the genetic and molecular bases underlying natural variation of reciprocal helping are scarce (not to say absent) despite the role of positive plant–plant interactions on overyielding in crop mixtures and in regulating the composition of natural plant communities (Brooker *et al.*, 2008; Li *et al.*, 2014; Wendling *et al.*, 2017). Based on an innovative strategy recently developed for global genome-to-genome analysis and employed in the human-HIV pathosystem (Bartha *et al.*, 2013), we propose an ecological genomics strategy of GWA mapping to identify natural genetic variants underlying mutualism between two plant species, without the need to obtain large phenotypic data sets. The strategy is composed of four steps (Figure 4(a)). The first step consists in collecting a substantial number (>100) of paired genotypes (one per species) across a specific geographic area. The second step would be testing for mutualism based on a small number of paired genotypes, i.e. whether the genotype from species A sampled in community X has a better performance in presence of the genotype from species B sampled from the same community than when growing alone or when growing with other genotypes from species B sampled in other communities. If mutualism between the two species is confirmed, the third step would include generating paired plant–plant genomic data, which will be facilitated by ever-cheaper genome-sequencing technologies. The fourth step would then comprise of performing joint association mapping analysis using both plant genomes in order to identify genetic markers in strong linkage disequilibrium across the two genomes. Based on co-evolutionary processes, this strategy of joint GWA mapping should allow description of the adaptive molecular landscape underlying mutualism between two plant species.

### Monospecific interactions versus plurispecific interactions versus diffuse biotic interactions within plant communities

Most studies reporting the genetic and molecular mechanisms of natural plant–plant interactions are based on monospecific heterospecific interactions (Figure 2). However, a focal plant rarely interacts with only one neighbor species, either in crop fields or in more natural environments. Instead, a focal plant interacts simultaneously with multiple plant partners belonging to several species. This strengthens the need to study plant–plant interactions in a community context. One may wonder whether the phenotypic response of a focal plant to plurispecific interaction results from the additivity of the individual phenotypic responses to monospecific interactions. Similarly, it remains to be tested whether QTLs of competitive

responses of a focal plant in a plurispecific neighborhood correspond to the sum of QTLs that are specific to a neighbor species and/or to the emergence of new QTLs (Figure 4(b)). The approach for evaluating the operation of plurispecific interactions between more than two plant species will require the evaluation of the performance of numerous genetic lines in all two-way, three-way and so on combinations. For the experiment to be feasible, such an evaluation can only be achieved by considering a reasonable number of interacting species (i.e. three or four species), which will however still be less than the number of plant species that a focal species may encounter in its natural communities (up to 89 species; Wilson *et al.*, 2012). To resolve this issue, a Genome Environment Analysis (GEA) approach can be used to identify the genetic and molecular basis associated with the interaction of a focal species with multiple and simultaneous interactors in plant communities. This approach will require: (i) the characterization of the plant communities associated with a given focal species; (ii) the genome sequencing of the focal species within each plant community; and (iii) statistical analyses aimed at identifying genomic regions of the focal species associated with descriptors of plant communities such as richness, alpha-diversity and composition. While GEA has been proved to successfully identify the genetic bases of adaptation to climate in plants (Hancock *et al.*, 2011), its power to identify the adaptive genetic bases to diffuse and complex biotic conditions remains to be tested. Nevertheless, by exploring diffuse biotic plant–plant interactions, the lofty goal of identifying adaptive QTLs associated with plant community descriptors can help to understand the role of community-wide selection.

### ACKNOWLEDGEMENTS

This work was funded by a PhD fellowship from the LABEX TULIP to HJS (ANR-10-LABX-41, ANR-11-IDEX-0002-02) and a PhD fellowship from the University of Toulouse to CL. This work was also supported by the Région Midi-Pyrénées (CLIMARES project) and the INRA Santé des Plantes et Environnement department (RESURRECTION project). SR is supported by a Starting Grant of the European Research Council (ERC-StG-336808). The authors declare no conflict of interest.

### SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

**Data Set S1.** List of the phenotypic traits measured in the QTL mapping studies.

### REFERENCES

- Addisu, M., Snape, J.W., Simmonds, J.R. and Gooding, M.J. (2009) Reduced height (*Rht*) and photoperiod insensitivity (*Ppd*) allele associations with establishment and early growth of wheat in contrasting production systems. *Euphytica*, **166**, 249–267.

- Adebesin, F., Widhalm, J.R., Boachon, B. et al. (2017) Emission of volatile organic compounds from petunia flowers is facilitated by an ABC transporter. *Science*, **356**(6345), 1386–1388.
- Ahmad-Ramli, M.F., Cornulier, T. and Johnson, D. (2013) Partitioning of soil phosphorus regulates competition between *Vaccinium vitis-idaea* and *Deschampsia cespitosa*. *Ecol. Evol.* **3**(12), 4243–4252.
- Aibara, I. and Miwa, K. (2014) Strategies for optimization of mineral nutrient transport in plants: multilevel regulation of nutrient-dependent dynamics of root architecture and transporter activity. *Plant Cell Physiol.* **55**(12), 2027–2036.
- Allen, B., Nowak, M.A. and Wilson, E.O. (2013) Limitations of inclusive fitness. *Proc. Natl Acad. Sci. USA*, **110**(50), 20135–20139.
- Asif, M., Yang, R.C., Navabi, A. et al. (2015) Mapping QTL, selection differentials, and the effect of *Rht-B1* under organic and conventionally managed systems in the Attila × CDC Go spring wheat mapping population. *Crop Sci.* **55**(3), 1129–1142.
- Bachelet, D., Neilson, R.P., Lenihan, J.M. and Drapek, R.J. (2001) Climate change effects on vegetation distribution and carbon budget in the United States. *Ecosystems*, **4**, 164–185.
- Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S. and Vivanco, J.M. (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.* **57**(1), 233–266.
- Baldwin, I.T., Halitschke, R., Paschold, A., von Dahl, C.C. and Preston, C.A. (2006) Volatile signaling in plant–plant interactions: ‘talking trees’ in the genomics era. *Science*, **311**(5762), 812–815.
- Baldwin, A.H., Jensen, K. and Schönfeldt, M. (2014) Warming increases plant biomass and reduces diversity across continents, latitudes, and species migration scenarios in experimental wetland communities. *Glob. Change Biol.* **20**(3), 835–850.
- Ballaré, C.L. (2014) Light regulation of plant defense. *Annu. Rev. Plant Biol.* **65**(1), 335–363.
- Ballaré, C.L. and Pierik, R. (2017) The shade-avoidance syndrome: multiple signals and ecological consequences. *Plant, Cell Environ.* **40**, 2530–2543.
- Balzerque, C., Darteville, T., Godon, C. et al. (2017) Low phosphate activates STOP1-ALMT1 to rapidly inhibit root cell elongation. *Nat. Commun.* **8**, 15300.
- Baron, E., Richirt, J., Villoutreix, R., Amsellem, L. and Roux, F. (2015) The genetics of intra- and interspecific competitive response and effect in a local population of an annual plant species. *Funct. Ecol.* **29**(10), 1361–1370.
- Bartelheimer, M., Schmid, C., Storf, J., Hell, K. and Bauer, S. (2015) Interspecific competition in *Arabidopsis thaliana*: a knowledge gap is starting to close. In *Progress in Botany* (Lüttge, U. and Beyschlag, W., eds). Vol. 76. Berlin: Springer, pp. 303–319.
- Bartha, I., Carlson, J.M., Brumme, C.J. et al. (2013) A genome-to-genome analysis of associations between human genetic variation, HIV-1 sequence diversity, and viral control. *eLife*, **2**, 1–16.
- Bartoli, C. and Roux, F. (2017) Genome-Wide Association studies in plant pathosystems: toward an ecological genomics approach. *Front. Plant Sci.* **8**, 763.
- Basu, C., Halfhill, M.D., Mueller, T.C. and Stewart, C.N. (2004) Weed genomics: new tools to understand weed biology. *Trends Plant Sci.* **9**(8), 391–398.
- Bates, T.R. and Lynch, J.P. (2001) Root hairs confer a competitive advantage under low phosphorus availability. *Plant Soil*, **236**(2), 243–250.
- Bennett, J.A., Maherali, H., Reinhart, K.O., Lekberg, Y., Hart, M.M. and Klironomos, J. (2017) Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. *Science*, **355**(6321), 181–184.
- Berendsen, R.L., Pieterse, C.M.J. and Bakker, P.A.H.M. (2012) The rhizosphere microbiome and plant health. *Trends Plant Sci.* **17**(8), 478–486.
- Bergelson, J. and Roux, F. (2010) Towards identifying genes underlying ecologically relevant traits in *Arabidopsis thaliana*. *Nat. Rev. Genet.* **11**(12), 867–879.
- Bertness, M.D. and Callaway, R. (1994) Positive interactions in communities. *Trends Ecol. Evol.* **9**(5), 187–191.
- Bever, J.D., Dickie, I.A., Facelli, E. et al. (2011) Rooting theories of plant community ecology in microbial interactions. *Trends Ecol. Evol.* **20**(8), 468–478.
- Biedrzycki, M.L., Lakshmanan, V. and Bais, H.P. (2011a) Transcriptome analysis of *Arabidopsis thaliana* plants in response to kin and stranger recognition. *Plant Signal Behav.* **6**(10), 1515–1524.
- Biedrzycki, M.L., Lakshmanan, V. and Bais, H.P. (2011b) The role of ABC transporters in kin recognition in *Arabidopsis thaliana*. *Plant Signal Behav.* **6**(8), 1154–1161.
- Bonsler, S.P. (2013) High reproductive efficiency as an adaptive strategy in competitive environments. *Funct. Ecol.* **27**(4), 876–885.
- Botto, J.F. and Coluccio, M.P. (2007) Seasonal and plant-density dependency for quantitative trait loci affecting flowering time in multiple populations of *Arabidopsis thaliana*. *Plant, Cell Environ.* **30**(11), 1465–1479.
- Boukar, O., Kong, L., Singh, B.B., Murdock, L. and Ohm, H.W. (2004) AFLP and AFLP-derived SCAR markers associated with *Striga gesnerioides* resistance in cowpea. *Crop Sci.* **44**(4), 1259–1264.
- Bowsher, A.W., Shetty, P., Anacker, B.L., Siefert, A., Strauss, S.Y. and Friesen, M.L. (2017) Transcriptomic responses to conspecific and congeneric competition in co-occurring *Trifolium*. *J. Ecol.* **105**(3), 602–615.
- Brachi, B., Aimé, C., Glorieux, C., Cuguen, J. and Roux, F. (2012) Adaptive value of phenological traits in stressful environments: predictions based on seed production and laboratory natural selection. *PLoS ONE*, **7**(3).
- Brachi, B., Villoutreix, R., Faure, N. et al. (2013) Investigation of the geographical scale of adaptive phenological variation and its underlying genetics in *Arabidopsis thaliana*. *Mol. Ecol.* **22**(16), 4222–4240.
- Brooker, R.W. and Callaghan, T.V. (1998) The Balance between positive and negative plant interactions and its relationship to environmental gradients: a model. *Oikos*, **81**(1), 196–207.
- Brooker, R.W., Maestre, F.T., Callaway, R.M. et al. (2008) Facilitation in plant communities: the past, the present, and the future. *J. Ecol.* **96**(1), 18–34.
- Brooker, R.W., Bennett, A.E., Cong, W.F. et al. (2015) Improving intercropping: a synthesis of research in agronomy, plant physiology and ecology. *New Phytol.* **206**(1), 107–117.
- Broz, A.K., Manter, D.K., Callaway, R.M., Paschke, M.W. and Vivanco, J.M. (2008) A molecular approach to understanding plant–plant interactions in the context of invasion biology. *Funct. Plant Biol.* **35**(11), 1123–1134.
- Bruno, J.F., Stachowicz, J.J. and Bertness, M.D. (2003) Inclusion of facilitation into ecological theory. *Trends Ecol. Evol.* **18**, 119–125.
- Bukowski, A.R. and Petermann, J.S. (2014) Intraspecific plant-soil feedback and intraspecific overyielding in *Arabidopsis thaliana*. *Ecol. Evol.* **4**(12), 2533–2545.
- Cahill, J.F. Jr, Kembel, S.W. and Gustafson, D.J. (2005) Differential genetic influences on competitive effect and response in *Arabidopsis thaliana*. *J. Ecol.* **93**(5), 958–967.
- Callaway, R.M. (1995) Positive interactions among plants. *Bot. Rev.* **61**(4), 306–349.
- Cardoso, C., Zhang, Y., Jamil, M. et al. (2014) Natural variation of rice strigolactone biosynthesis is associated with the deletion of two *MAX1* orthologs. *Proc. Natl Acad. Sci. USA*, **111**(6), 2379–2384.
- Cecchini, N., Steffes, K., Schläppi, M.R., Gifford, A.N. and Greenberg, J.T. (2015) *Arabidopsis* AZI1 family proteins mediate signal mobilization for systemic defence priming. *Nat. Commun.* **6**, 7658.
- Cerrudo, I., Caliri-Ortiz, M.E., Keller, M.M., Degano, M.E., Demkura, P.V. and Ballaré, C.L. (2017) Exploring growth-defence trade-offs in *Arabidopsis*: phytochrome B inactivation requires JAZ10 to suppress plant immunity but not to trigger shade-avoidance responses. *Plant, Cell Environ.* **40**, 635–644.
- Chehab, E.W., Yao, C., Henderson, Z., Kim, S. and Braam, J. (2012) *Arabidopsis* touch-induced morphogenesis is jasmonate mediated and protects against pests. *Curr. Biol.* **22**(8), 701–706.
- Chesson, P. (2000) Mechanism of maintenance of species diversity. *Annu. Rev. Ecol. Syst.* **31**, 343–366.
- Choe, E., Drnevich, J. and Williams, M.M. (2016) Identification of crowding stress tolerance co-expression networks involved in sweet corn yield. *PLoS ONE*, **11**(1), 1–20.
- Cipollini, D.F. (2002) Does competition magnify the fitness costs of induced responses in *Arabidopsis thaliana*? A manipulative approach. *Oecologia*, **131**(4), 514–520.
- Clements, D.R., Dittmas, A. and Hyvönen, T. (2014) Ecology and management of weeds in a changing climate. In *Recent Advances in Weed Management* (Chauhan, B.S. and Mahajan, G., eds). New York: Springer Science + Business Media, pp. 13–38.
- Coleman, R., Gill, G. and Rebetzke, G. (2001) Identification of quantitative trait loci for traits conferring weed competitiveness in wheat (*Triticum aestivum* L.). *Aust J Agric Res.* **52**, 1235–1246.

- Crepney, M.A. and Casal, J.J. (2015) Photoreceptor-mediated kin recognition in plants. *New Phytol.* **205**(1), 329–338.
- Crutsinger, G.M., Collins, M.D., Fordyce, J.A., Gompert, Z., Nice, C.C. and Sanders, N.J. (2006) Plant genotypic diversity predicts community structure and governs an ecosystem process. *Science*, **313**(5789), 966–968.
- van Dam, N.M. and Bouwmeester, H.J. (2016) Metabolomics in the rhizosphere: tapping into belowground chemical communication. *Trends Plant Sci.* **21**(3), 256–265.
- Depuydt, S. (2014) Arguments for and against self and non-self root recognition in plants. *Front. Plant Sci.* **5**, 1–7.
- Díaz-Ruiz, R., Torres, A., Gutierrez, M.V. et al. (2009) Mapping of quantitative trait loci controlling *Orobanche foetida* Poir. resistance in faba bean (*Vicia faba* L.). *Afr. J. Biotechnol.* **8**(12), 2718–2724.
- Díaz-Ruiz, R., Torres, A.M., Satovic, Z., Gutierrez, M.V., Cubero, J.I. and Román, B. (2010) Validation of QTLs for *Orobanche crenata* resistance in faba bean (*Vicia faba* L.) across environments and generations. *Theor. Appl. Genet.* **120**(5), 909–919.
- Dicke, M. and Baldwin, I.T. (2010) The evolutionary context for herbivore-induced plant volatiles: beyond ‘cry for help’. *Trends Plant Sci.* **15**(3), 167–175.
- Ding, X., Yang, M., Huang, H. et al. (2015) Priming maize resistance by its neighbors: activating 1,4-benzoxazine-3-ones synthesis and defense gene expression to alleviate leaf disease. *Front. Plant Sci.* **6**(October), 1–11.
- Dita, M.A., Die, J.V., Román, B. et al. (2009) Gene expression profiling of *Medicago truncatula* roots in response to the parasitic plant *Orobanche crenata*. *Weed Res.* **49**(suppl. 1), 66–80.
- Dudley, S.A. (2015) Plant cooperation. *AoB Plants*, **7**, plv113.
- Ebana, K., Yan, W., Dilday, R.H., Namai, H. and Okuno, K. (2001) Analysis of QTL associated with the allelopathic effect of rice using water-soluble extracts. *Breed. Sci.* **51**, 47–51.
- Fang, S., Clark, R.T., Zheng, Y. et al. (2013) Genotypic recognition and spatial responses by rice roots. *Proc. Natl Acad. Sci. USA*, **110**(7), 2670–2675.
- Filialt, D.L., Wessinger, C.A., Dinneny, J.R. et al. (2008) Amino acid polymorphisms in *Arabidopsis* phytochrome B cause differential responses to light. *Proc. Natl Acad. Sci. USA*, **105**(8), 3157–3162.
- Fitter, A., Williamson, L., Linkohr, B. and Leyser, O. (2002) Root system architecture determines fitness in an *Arabidopsis* mutant in competition for immobile phosphate ions but not for nitrate ions. *Hungarian Quarterly*, **49**(191), 2017–2022.
- Fondevilla, S., Fernández-Aparicio, M., Satovic, Z., Emeran, A.A., Torres, A.M., Moreno, M.T. and Rubiales, D. (2010) Identification of quantitative trait loci for specific mechanisms of resistance to *Orobanche crenata* Forsk. in pea (*Pisum sativum* L.). *Mol. Breeding*, **25**(2), 259–272.
- Frachon, L., Libourel, C., Villoutreix, R. et al. (2017) Intermediate degrees of synergistic pleiotropy drive adaptive evolution in ecological time. *Nat. Ecol. Evol.* **1**, 1551–1561.
- Frank, D.A., Pontes, A.W., Maine, E.M., Caruana, J., Raina, R., Raina, S. and Fridley, J.D. (2010) Grassland root communities: Species distributions and how they are linked to aboveground abundance. *Ecology*, **91**(11), 3201–3209.
- Fraser, D.P., Hayes, S. and Franklin, K.A. (2016) Photoreceptor crosstalk in shade avoidance. *Curr. Opin. Plant Biol.* **33**(Supplement C), 1–7.
- Fridborg, I., Kuusk, S., Moritz, T. and Sundberg, E. (1999) The *Arabidopsis* dwarf mutant shi exhibits reduced gibberellin responses conferred by overexpression of a new putative zinc finger protein. *Plant Cell*, **11**(6), 1019–1032.
- Fu, X., Wu, X., Zhou, X., Liu, S., Shen, Y. and Wu, F. (2015) Companion cropping with potato onion enhances the disease resistance of tomato against *Verticillium dahliae*. *Front. Plant Sci.* **6**(September), 1–13.
- Gao, X., Wu, M., Xu, R., Wang, X., Pan, R., Kim, H.-J. and Liao, H. (2014) Root interactions in a maize/soybean intercropping system control soybean soil-borne disease, Red Crown Rot. *PLOS ONE*, **9**(5), e95031.
- Garbeva, P., Postma, J., Van Veen, J.A. and Van Elsas, J.D. (2006) Effect of above-ground plant species on soil microbial community structure and its impact on suppression of *Rhizoctonia solani* AG3. *Environ. Microbiol.* **8**(2), 233–246.
- Geisler, M., Gibson, D.J., Lindsey, K.J., Millar, K. and Wood, A.J. (2012) Upregulation of photosynthesis genes, and downregulation of stress defense genes, is the response of *Arabidopsis thaliana* shoots to intraspecific competition. *Bot. Stud.* **53**(1), 85–96.
- Gilman, S.E., Urban, M.C., Tewksbury, J., Gilchrist, G.W. and Holt, R.D. (2010) A framework for community interactions under climate change. *Trends Ecol. Evol.* **25**(6), 325–331.
- Gobena, D., Shimels, M., Rich, P.J. et al. (2017) Mutation in sorghum *LOW GERMINATION STIMULANT 1* alters strigolactones and causes *Striga* resistance. *Proc. Natl Acad. Sci. USA*, **114**(17), 4471–4476.
- Goldberg, D.E. and Barton, A.M. (1992) Patterns and consequences of interspecific competition in natural communities - a review of field experiments with plants. *Am. Nat.* **139**(4), 771–801.
- Gommers, C.M.M., Keuskamp, D.H., Buti, S. et al. (2017) Molecular profiles of contrasting shade response strategies in wild plants: differential control of immunity and shoot elongation. *Plant Cell*, **29**(2), 331–344.
- Gonzalo, M., Vyn, T.J., Holland, J.B. and McIntyre, L.M. (2006) Mapping density response in maize: a direct approach for testing genotype and treatment interactions. *Genetics*, **173**(1), 331–348.
- Gonzalo, M., Holland, J.B., Vyn, T.J. and McIntyre, L.M. (2010) Direct mapping of density response in a population of B73 × Mo17 recombinant inbred lines of maize (*Zea mays* L.). *Heredity*, **104**(6), 583–599.
- Goodwin, S., McPherson, J.D. and McCombie, W.R. (2016) Coming of age: ten years of next-generation sequencing technologies. *Nat. Rev. Genet.* **17**(6), 333–351.
- Granberry, M.C. (2016) Identifying Quantitative Trait Loci (QTL) associated with suppression of Italian Ryegrass in an AGS 2000 × NC-Neuse Soft Red Winter wheat population. PhD Thesis.
- Groner, M.G. (1974) Intraspecific allelopathy in *Kalanchoe daigremontiana*. *Bot. Gaz.* **135**(1), 73–79.
- Gundel, P.E., Pierik, R., Mommer, L. and Ballaré, C.L. (2014) Competing neighbors: light perception and root function. *Oecologia*, **176**(1), 1–10.
- Guo, J., Chen, Z., Liu, Z. et al. (2011) Identification of genetic factors affecting plant density response through QTL mapping of yield component traits in maize (*Zea mays* L.). *Euphytica*, **182**(3), 409–422.
- Gurney, A.L., Slate, J., Press, M.C. and Scholes, J.D. (2006) A novel form of resistance in rice to the angiosperm parasite *Striga hermonthica*. *New Phytol.* **169**(1), 199–208.
- Gutiérrez, N., Palomino, C., Satovic, Z. et al. (2013) QTLs for *Orobanche* spp. resistance in faba bean: Identification and validation across different environments. *Mol. Breeding*, **32**(4), 909–922.
- Halty, V., Valdés, M., Tejera, M., Picasso, V. and Fort, H. (2017) Modelling plant interspecific interactions from experiments of perennial crop mixtures to predict optimal combinations. *Ecol. Appl.* **27**, 2277–2289.
- Hancock, A.M., Brachi, B., Faure, N. et al. (2011) *Arabidopsis thaliana* genome. *Science*, **334**, 83–86.
- Hausmann, B.I.G., Hess, D.E., Omany, G.O. et al. (2004) Genomic regions influencing resistance to the parasitic weed *Striga hermonthica* in two recombinant inbred populations of sorghum. *Theor. Appl. Genet.* **109**(5), 1005–1016.
- Hermisson, J. and Pennings, P.S. (2005) Soft sweeps: molecular population genetics of adaptation from standing genetic variation. *Genetics*, **169**, 2335–2352.
- Hooper, A.M., Caulfield, J.C., Hao, B., Pickett, J.A., Midega, C.A.O. and Khan, Z.R. (2015) Isolation and identification of *Desmodium* root exudates from drought tolerant species used as intercrops against *Striga hermonthica*. *Phytochemistry*, **117**, 380–387.
- Horvath, D.P., Gulden, R. and Clay, S.A. (2006) Microarray analysis of late-season velvetleaf (*Abutilon theophrasti*) impact on corn. *Weed Sci.* **54**, 983–994.
- Horvath, D.P., Hansen, S.A., Moriles-Miller, J.P. et al. (2015) RNAseq reveals weed-induced PIF3-like as a candidate target to manipulate weed stress response in soybean. *New Phytol.* **207**(1), 196–210.
- Huang, K., Mellor, K.E., Paul, S.N., Lawson, M.J., Mackey, A.J. and Timko, M.P. (2012) Global changes in gene expression during compatible and incompatible interactions of cowpea (*Vigna unguiculata* L.) with the root parasitic angiosperm *Striga gesnerioides*. *BMC Genom.* **13**(1), 402.
- Huang, X., Ding, J., Effgen, S., Turck, F. and Koornneef, M. (2013) Multiple loci and genetic interactions involving flowering time genes regulate stem branching among natural variants of *Arabidopsis*. *New Phytol.* **199**(3), 843–857.

- Hwang, J.U., Song, W.Y., Hong, D. et al. (2016) Plant ABC transporters enable many unique aspects of a terrestrial plant's lifestyle. *Molecular Plant*, **9**(3), 338–355.
- Imerovski, I., Dimitrijević, A., Miladinović, D., Dedić, B., Jocić, S., Tubić, N.K. and Cvejić, S. (2016) Mapping of a new gene for resistance to broomrape races higher than F. *Euphytica*, **209**(2), 281–289.
- Isaac, M. and Anglaere, L.C.N. (2013) An *in situ* approach to detect tree root ecology: linking ground-penetrating radar imaging to isotope-derived water acquisition zones. *Ecol. Evol.* **3**, 1330–1339.
- Isbell, F., Calcagno, V., Hector, A. et al. (2011) High plant diversity is needed to maintain ecosystem services. *Nature*, **477**(7363), 199–202.
- Jensen, L.B., Courtois, B., Shen, L., Li, Z., Olofsson, M. and Mauleon, R.P. (2001) Locating genes controlling allelopathic effects against barnyard-grass in upland rice. *Agron. J.* **93**, 21–26.
- Jensen, L.B., Courtois, B. and Olofsson, M. (2008) Quantitative trait loci analysis of allelopathy in rice. *Crop Sci.* **48**(4), 1459–1469.
- Johnson, M.G., Tingey, D.T., Phillips, D.L. and Storm, M.J. (2001) Advancing fine root research with minirhizotrons. *Environ. Exp. Bot.* **45**, 263–289.
- Johnson, M.T.J., Lajeunesse, M. and Agrawal, A.A. (2006) Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. *Ecol. Lett.* **9**(1), 24–34.
- Jones, F.A., Erickson, D.L., Bernal, M.A. et al. (2011) The roots of diversity: below ground species richness and rooting distributions in a tropical forest revealed by DNA barcodes and inverse modeling. *PLoS ONE*, **6**(9), e24506.
- Joseph, B., Lau, L. and Kliebenstein, D.J. (2015) Quantitative variation in responses to root spatial constraint within *Arabidopsis thaliana*. *Plant Cell*, **27**(8), 2227–2243.
- Kaewchumng, K. and Price, A.H. (2008) A study on the susceptibility of rice cultivars to *Striga hermonthica* and mapping of *Striga* tolerance quantitative trait loci in rice. *New Phytol.* **180**(1), 206–216.
- Kang, J., Park, J., Choi, H., Burla, B., Kretschmar, T., Lee, Y. and Martinoia, E. (2011) Plant ABC transporters. *The Arabidopsis Book*, **9**, e0153.
- Karban, R., Yang, L.H. and Edwards, K.F. (2014) Volatile communication between plants that affects herbivory: a meta-analysis. *Ecol. Lett.* **17**(1), 44–52.
- Kesanakurti, P.R., Fazekas, A.J., Burgess, K.S. et al. (2011) Spatial patterns of plant diversity below-ground as revealed by DNA barcoding. *Mol. Ecol.* **20**(6), 1289–1302.
- Khanh, T.D., Xuan, T.D. and Chung, I.M. (2007) Rice allelopathy and the possibility for weed management. *Ann. Appl. Biol.* **151**(3), 325–339.
- Kikuchi, S., Bheemanahalli, R., Jagadish, K.S.V. et al. (2017) Genome-wide association mapping for phenotypic plasticity in rice. *Plant, Cell Environ.* **40**(8), 1565–1575.
- Kretschmar, T., Burla, B., Lee, Y., Martinoia, E. and Nagy, R. (2011) Functions of ABC transporters in plants. *Essays Biochem.* **50**, 145–160.
- Ku, L., Zhang, L., Tian, Z. et al. (2015) Dissection of the genetic architecture underlying the plant density response by mapping plant height-related traits in maize (*Zea mays* L.). *Mol. Genet. Genomics*, **290**(4), 1223–1233.
- Ku, L., Ren, Z., Chen, X. et al. (2016) Genetic analysis of leaf morphology underlying the plant density response by QTL mapping in maize (*Zea mays* L.). *Mol. Breeding*, **36**(5), 1–16.
- Latz, E., Eisenhauer, N., Scheu, S. and Jousset, A. (2015) Plant identity drives the expression of biocontrol factors in a rhizosphere bacterium across a plant diversity gradient. *Funct. Ecol.* **29**(9), 1225–1234.
- Latz, E., Eisenhauer, N., Rall, B.C., Scheu, S. and Jousset, A. (2016) Unravelling linkages between plant community composition and the pathogen-suppressive potential of soils. *Sci. Rep.* **6**(1), 23584.
- Lebeis, S.L., Paredes, S.H., Lundberg, D.S. et al. (2015) Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. *Science*, **349**(6250), 860–864.
- Lee, S., Seo, K.I., Koo, J.H., Hur, H.S. and Shin, J.C. (2005) QTLs and molecular markers associated with rice allelopathy. 4th World Congress on Allelopathy. The Regional Institute Ltd, p. 21–26.
- Lee, H., Gurtowski, J., Yoo, S. et al. (2016) Third-generation sequencing and the future of genomics. *bioRxiv*. 48603.
- Li, J. and Timko, M.P. (2009) Gene-for-Gene resistance in *Striga*-Cowpea associations. *Science*, **325**(5944), 1094–1094.
- Li, L., Tilman, D., Lambers, H. and Zhang, F.S. (2014) Plant diversity and overyielding: Insights from belowground facilitation of intercropping in agriculture. *New Phytol.* **203**(1), 63–69.
- Li, B., Li, Y.-Y., Wu, H.-M. et al. (2016) Root exudates drive interspecific facilitation by enhancing nodulation and N<sub>2</sub> fixation. *Proc. Natl Acad. Sci. USA*, **113**(23), 6496–6501.
- Litrice, I. and Violle, C. (2015) Diversity in plant breeding: a new conceptual framework. *Trends Plant Sci.* **20**(10), 604–613.
- Lobet, G., Pagès, L. and Draye, X. (2011) A novel image-analysis toolbox enabling quantitative analysis of root system architecture. *Plant Physiol.* **157**(1), 29–39.
- Lobet, G., Draye, X. and Périlleux, C. (2013) An online database for plant image analysis software tools. *Plant Methods*, **9**, 38.
- López Pereira, M., Sadras, V.O., Batista, W., Casal, J.J. and Hall, A.J. (2017) Light-mediated self-organization of sunflower stands increases oil yield in the field. *Proc. Natl Acad. Sci. USA*, **114**(30), 7975–7980.
- Loreau, M. and de Mazancourt, C. (2013) Biodiversity and ecosystem stability: a synthesis of underlying mechanisms. *Ecol. Lett.* **16**, 106–115.
- Louarn, J., Boniface, M.-C., Pouilly, N., Velasco, L., Pérez-Vich, B., Vincourt, P. and Muñoz, S. (2016) Sunflower resistance to broomrape (*Orobancha cumana*) is controlled by specific QTLs for different parasitism stages. *Front. Plant Sci.* **7**, 1–14.
- Lu, Y.H., Melero-Vara, J.M., García-Tejada, J.A. and Blanchard, P. (2000) Development of SCAR markers linked to the gene Or5 conferring resistance to broomrape (*Orobancha cumana* Wallr.) in sunflower. *Theor. Appl. Genet.* **100**(3), 625–632.
- Markovic, D., Nikolic, N., Glinwood, R., Seisenbaeva, G. and Ninkovic, V. (2016) Plant responses to brief touching: a mechanism for early neighbour detection? *PLoS ONE*, **11**(11), e0165742.
- Masclaux, F.G., Bruessow, F., Schweizer, F., Gouhier-Darimont, C., Keller, L. and Reymond, P. (2012) Transcriptome analysis of intraspecific competition in *Arabidopsis thaliana* reveals organ-specific signatures related to nutrient acquisition and general stress response pathways. *BMC Plant Biol.* **12**(1), 227.
- Massalha, H., Korenblum, E., Tholl, D. and Aharoni, A. (2017) Small molecules below-ground: the role of specialized metabolites in the rhizosphere. *Plant J.* **90**, 788–807.
- Mbengue, M., Navaud, O., Peyraud, R. et al. (2016) Emerging trends in molecular interactions between plants and the broad host range fungal pathogens *Botrytis cinerea* and *Sclerotinia sclerotiorum*. *Front. Plant Sci.* **31**, 422.
- Michaud, O., Fiorucci, A., Xenarios, I. and Fankhauser, C. (2017) Local auxin production underlies a spatially restricted neighbor-detection response in *Arabidopsis*. *Proc. Natl Acad. Sci. USA*, **114**(28), 7444–7449.
- Mommer, L., Wagemaker, C.A.M., De Kroon, H. and Ouborg, N.J. (2008) Unravelling below-ground plant distributions: a real-time polymerase chain reaction method for quantifying species proportions in mixed root samples. *Mol. Ecol. Resour.* **8**(5), 947–953.
- Mommer, L., Dumbrell, A.J., Wagemaker, C.A.M. and Ouborg, N.J. (2011) Belowground DNA-based techniques: untangling the network of plant root interactions. *Plant Soil*, **348**(1–2), 115–121.
- Mommer, L., Kirkegaard, J. and van Ruijven, J. (2016) Root-Roost interactions: towards a rhizosphere framework. *Trends Plant Sci.* **21**(3), 209–217.
- Moncada, P., Martínez, C.P., Borrero, J. et al. (2001) Quantitative trait loci for yield and yield components in an *Oryza sativa* × *Oryza rufipogon* BC 2 F 2 population evaluated in an upland environment. *Theor. Appl. Genet.* **102**(1), 41–52.
- Mönchgesang, S., Strehmel, N., Schmidt, S. et al. (2016) Natural variation of root exudates in *Arabidopsis thaliana*-linking metabolomic and genomic data. *Sci. Rep.* **6**(1), 29033.
- Mora-Macias, J., Ojeda-Rivera, J.O., Gutiérrez-Alanís, D. et al. (2017) Malate-dependent Fe accumulation is a critical checkpoint in the root developmental response to low phosphate. *Proc. Natl Acad. Sci.* **114**(17), E3563–E3572.
- Moriles, J., Hansen, S., Horvath, D.P., Reicks, G., Clay, D.E. and Clay, S.A. (2012) Microarray and growth analyses identify differences and similarities of early corn response to weeds, shade, and nitrogen stress. *Weed Sci.* **60**(2), 158–166.
- Mutic, J.J. and Wolf, J.B. (2007) Indirect genetic effects from ecological interactions in *Arabidopsis thaliana*. *Mol. Ecol.* **16**(11), 2371–2381.
- Mutuku, J.M., Yoshida, S., Shimizu, T. et al. (2015) The WRKY45-dependent signaling pathway is required for resistance against *Striga hermonthica* parasitism. *Plant Physiol.* **168**, 1152–1163.

- Nagatani, A., Reed, J.W. and Chory, J. (1993) Isolation and initial characterization of *Arabidopsis* mutants that are deficient in Phytochrome A. *Plant Physiol.* **102**(1), 269–277.
- Neve, P., Vila-aiub, M. and Roux, F. (2009) Evolutionary-thinking in agricultural weed management. *Wiley*, **184**(4), 783–793.
- Ninkovic, V. (2003) Volatile communication between barley plants affects biomass allocation. *J. Exp. Bot.* **54**(389), 1931–1939.
- Ninkovic, V., Dahlin, I., Vucetic, A., Petrovic-Obradovic, O., Glinwood, R. and Webster, B. (2013) Volatile exchange between undamaged plants - a new mechanism affecting insect orientation in intercropping. *PLoS ONE*, **8**(7), e69431.
- Nowak, M.A., Tarnita, C.E. and Wilson, E.O. (2010) The evolution of eusociality. *Nature*, **466**, 1057–1062.
- O'Brien, J.A., Vega, A., Bouguyon, E., Krouk, G., Gojon, A., Coruzzi, G. and Gutiérrez, R.A. (2017) Nitrate transport, sensing, and responses in plants. *Molecular Plant*, **9**(6), 837–856.
- Orke, E.C. (2006) Crop losses to pests. *J. Agric. Sci.* **144**, 31–43.
- Okuno, K. and Ebana, K. (2003) Identification of QTL controlling allelopathic effects in rice: genetic approaches to biological control of weeds. *Jpn. Agric. Res. Q.* **37**(2), 77–81.
- Olofsdotter, M., Jensen, L.B. and Courtois, B. (2002) Improving crop competitive ability using allelopathy - an example from rice. *Plant Breeding*, **121**(1), 1–9.
- Orr, H.A. (2005) The genetic theory of adaptation: a brief history. *Nat. Rev. Genet.* **6**, 119–127.
- Ouédraogo, J.T., Maheshwari, V., Berner, D.K., St-Pierre, C.A., Belzile, F. and Timko, M.P. (2001) Identification of AFLP markers linked to resistance of cowpea (*Vigna unguiculata* L.) to parasitism by *Striga gesnerioides*. *Theor. Appl. Genet.* **102**(6–7), 1029–1036.
- Ouédraogo, J.T., Tignegre, J.-B., Timko, M.P. and Beizile, F.J. (2002) AFLP markers linked to resistance against *Striga gesnerioides* race 1 in cowpea (*Vigna unguiculata*). *Genome*, **45**(5), 787–793.
- Pakeman, R.J., Karley, A.J., Newton, A.C., Morcillo, L., Brooker, R.W. and Schöb, C. (2015) A trait-based approach to crop-weed interactions. *Eur. J. Agron.* **70**, 22–32.
- Pantazopoulou, C.K., Bongers, F.J., Küpers, J.J. et al. (2017) Neighbor detection at the leaf tip adaptively regulates upward leaf movement through spatial auxin dynamics. *Proc. Natl Acad. Sci. USA*, **114**(28), 7450–7455.
- Pearse, I.S., Porensky, L.M., Yang, L.H. et al. (2012) Complex consequences of herbivory and interplant cues in three annual plants. *PLoS ONE*, **7**(5), e38105.
- Peters, K., Breitsameter, L. and Gerowitz, B. (2014) Impact of climate change on weeds in agriculture: a review. *Agron. Sustain. Dev.* **34**(4), 707–721.
- Pierik, R., Mommer, L. and Voeseek, L.A. (2013) Molecular mechanisms of plant competition: neighbour detection and response strategies. *Funct. Ecol.* **27**(4), 841–853.
- Prieto, I., Violle, C., Barre, P., Durand, J.-L., Ghesquiere, M. and Litrico, I. (2015) Complementary effects of species and genetic diversity on productivity and stability of sown grasslands. *Nature Plants*, **1**(4), 15033.
- Reed, J.W., Nagpal, P., Poole, D.S., Furuya, M. and Chory, J. (1993) Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout *Arabidopsis* development. *Plant Cell*, **5**(2), 147–157.
- Reed, J.W., Nagatani, A., Elich, T.D., Fagan, M. and Chory, J. (1994) Phytochrome A and Phytochrome B have overlapping but distinct functions in *Arabidopsis* development. *Plant Physiol.* **104**(4), 1139–1149.
- Ren, L., Su, S., Yang, X., Xu, Y., Huang, Q. and Shen, Q. (2008) Intercropping with aerobic rice suppressed *Fusarium* wilt in watermelon. *Soil Biol. Biochem.* **40**(3), 834–844.
- Ren, L., Huo, H., Zhang, F., Hao, W., Xiao, L., Dong, C. and Xu, G. (2016) The components of rice and watermelon root exudates and their effects on pathogenic fungus and watermelon defense. *Plant Signal. Behav.* **11**(6), 1–9.
- Rice, E.L. (1984) *Allelopathy*, 2nd edn. Orlando: Academic Press.
- Riedlmeier, M., Ghirardo, A., Wenig, M. et al. (2017) Monoterpenes support systemic acquired resistance within and between plants. *Plant Cell*, **29**(6), 1440 LP–1459.
- Román, B., Torres, A.M.A., Rubiales, D., Cubero, J.I., Satovic, Z. and Román, B. (2002) Mapping of quantitative trait loci controlling broomrape (*Orobanche crenata* Forsk.) resistance in faba bean (*Vicia faba* L.). *Genome*, **45**, 1057–1063.
- Roux, F. and Bergelson, J. (2016) The genetics underlying natural variation in the biotic interactions of *Arabidopsis thaliana*: the challenges of linking evolutionary genetics and community ecology. *Curr. Top. Dev. Biol.*, **119**, 111–156.
- Roy, B.A. (1993) Floral mimicry by a plant pathogen. *Nature*, **362**(6415), 56–58.
- Satish, K., Gutema, Z., Grenier, C., Rich, P.J. and Ejeta, G. (2012) Molecular tagging and validation of microsatellite markers linked to the low germination stimulant gene (lgs) for *Striga* resistance in sorghum [*Sorghum bicolor* (L.) Moench]. *Theor. Appl. Genet.* **124**(6), 989–1003.
- Schmid, C., Bauer, S., Müller, B. and Bartelheimer, M. (2013) Belowground neighbor perception in *Arabidopsis thaliana* studied by transcriptome analysis: roots of *Hieracium pilosella* cause biotic stress. *Front. Plant Sci.* **4**(August), 1–17.
- Schmidt, D.D. and Baldwin, I.T. (2006) Transcriptional responses of *Solanum nigrum* to methyl jasmonate and competition: a glasshouse and field study. *Funct. Ecol.* **20**(3), 500–508.
- Schmitt, J., McCormac, A.C. and Smith, H. (1995) A test of the adaptive plasticity hypothesis using transgenic and mutant plants disabled in phytochrome-mediated elongation responses to neighbors. *Am. Nat.* **146**(6), 937.
- Schnee, C., Kollner, T.G., Held, M., Turlings, T.C.J., Gershenzon, J. and Degenhardt, J. (2006) The products of a single maize sesquiterpene synthase form a volatile defense signal that attracts natural enemies of maize herbivores. *Proc. Natl Acad. Sci. USA*, **103**(4), 1129–1134.
- Schuman, M.C., Allmann, S. and Baldwin, I.T. (2015) Plant defense phenotypes determine the consequences of volatile emission for individuals and neighbors. *eLife*, **4**(2008), 1–43.
- Selosse, M.-A., Richard, F., He, X. and Simard, S.W. (2006) Mycorrhizal networks: des liaisons dangereuses? *Trends Ecol. Evol.* **21**(11), 621–628.
- Semchenko, M., Saar, S. and Lepik, A. (2014) Plant root exudates mediate neighbour recognition and trigger complex behavioural changes. *New Phytol.* **204**(3), 631–637.
- Semchenko, M., Saar, S. and Lepik, A. (2017) Intraspecific genetic diversity modulates plant–soil feedback and nutrient cycling. *New Phytol.* **216**, 90–98.
- Shimizu, H. (2012) Developmental change of vegetative plant architecture of annual-form-wild rice (*Oryza rufipogon* Griff.) elevates competitive ability during the late development under a dense condition. *Am. J. Plant Sci.* **3**(5), 670–687.
- Shimizu, H., Maruoka, M., Ichikawa, N., Baruah, A.R., Uwatoko, N., Sano, Y. and Onishi, K. (2010) Genetic control of phenotypic plasticity in Asian cultivated and wild rice in response to nutrient and density changes. *Genome*, **53**(3), 211–223.
- Singer, A., Travis, J.M.J. and Johst, K. (2013) Interspecific interactions affect species and community responses to climate shifts. *Oikos*, **122**(3), 358–366.
- St. Pierre, S., Springer, N.M. and Muehlbauer, G.J. (2011) Density stress has minimal impacts on the barley or maize seedling transcriptome. *The Plant Genome*, **4**(1), 47–54.
- Sun, C.-H., Yu, J.-Q. and Hu, D.-G. (2017) Nitrate: a crucial signal during lateral roots development. *Front. Plant Sci.* **8**, 1–9.
- Swarbrick, P., Huang, K., Liu, G., Slate, J., Press, M. and Scholes, J. (2008) Global patterns of gene expression in rice cultivars undergoing a susceptible or resistant interaction with the parasitic plant *Striga hermonthica*. *New Phytol.* **179**, 515–529.
- Swarbrick, P.J., Scholes, J.D., Press, M.C. and Slate, J. (2009) A major QTL for resistance of rice to the parasitic plant *Striga hermonthica* is not dependent on genetic background. *Pest Manag. Sci.* **65**(5), 528–532.
- Tabata, R., Sumida, K., Yoshii, T., Ohshima, K., Shinohara, H. and Matsubayashi, Y. (2014) Perception of root-derived peptides by shoot LRR-RKs mediates systemic N-demand signaling. *Science*, **346**(6207), 343–346.
- Tang, S., Heesacker, A., Kishore, V.K., Fernandez, A., Sadik, E.S., Cole, G. and Knapp, S.J. (2003) Genetic mapping of the gene for resistance to race E in sunflower. *Crop Sci.* **43**(3), 1021–1028.
- Tessadori, F., van Zanten, M., Pavlova, P. et al. (2009) PHYTOCHROME B and HISTONE DEACETYLASE 6 control light-induced chromatin compaction in *Arabidopsis thaliana*. *PLoS Genet.* **5**(9), e1000638.

- Teste, F.P., Veneklaas, E.J., Dixon, K.W. and Lambers, H. (2014) Complementary plant nutrient-acquisition strategies promote growth of neighbour species. *Funct. Ecol.* **28**(4), 819–828.
- Teste, F.P., Kardol, P., Turner, B.L., Wardle, D.A., Zemunik, G., Renton, M. and Laliberté, E. (2017) Plant-soil feedback and the maintenance of diversity in Mediterranean-climate shrublands. *Science*, **355**(6321), 173–176.
- Till-Bottraud, I. and de Villemereuil, P. (2016) Kin recognition or phenotype matching? *New Phytol.* **209**(1), 13–14.
- Tilman, D. (1985) The resource ratio hypothesis of succession. *Am. Nat.* **125**, 827–852.
- Tilman, D., Lehman, C.L. and Thomson, K.T. (1997) Plant diversity and ecosystem productivity: theoretical considerations. *Proc. Natl Acad. Sci. USA*, **94**, 1857–1861.
- Tilman, D., Reich, P.B., Knops, J., Wedin, D., Mielke, T. and Lehman, C. (2001) Diversity and productivity in a long-term grassland experiment. *Science*, **294**, 843–845.
- Valderrama, M.R., Roman, B., Satovic, Z., Rubiales, D., Cubero, J.I. and Torres, A.M. (2004) Locating quantitative trait loci associated with *Orobanche crenata* resistance in pea. *Weed Res.* **44**(4), 323–328.
- Van de Putten, W.H., Bardgett, R.D., Bever, J.D. et al. (2013) Plant-soil feedback: the past, the present and future challenges. *J. Ecol.* **101**(2), 265–276.
- Vandenkoornhuysse, P., Quaiser, A., Duhamel, M., Le Van, A. and Dufresne, A. (2015) The importance of the microbiome of the plant holobiont. *New Phytol.* **206**, 1196–1206.
- Walder, F., Niemann, H., Natarajan, M., Lehmann, M.F., Boller, T. and Wiemken, A. (2012) Mycorrhizal networks: common goods of plants shared under unequal terms of trade. *Plant Physiol.* **159**(2), 789–797.
- Weiner, J., Andersen, S.B., Wille, W.K.M., Griepentrog, H.W. and Olsen, J.M. (2010) Evolutionary agroecology: the potential for cooperative, high density, weed-suppressing cereals. *Evol. Appl.* **3**(5–6), 473–479.
- Wendling, M., Büchi, L., Amossé, C., Jeangros, B., Walter, A. and Charles, R. (2017) Specific interactions leading to transgressive overyielding in cover crop mixtures. *Agr. Ecosyst. Environ.* **241**, 88–99.
- Whitham, T.G., Bailey, J.K., Schweitzer, J.A. et al. (2006) A framework for community and ecosystem genetics: from genes to ecosystems. *Nat. Rev. Genet.* **7**(7), 510–523.
- Williams, M.E. (2013) Plants are not alone. Teaching tools in plant biology: Lecture notes. *Plant Cell* (Online). <https://doi.org/10.1105/tpc.111.tt0611>.
- Wilson, J.B., Peet, R.K., Dengler, J. and Pärtel, M. (2012) Plant species richness: the world records. *J. Veg. Sci.* **23**(4), 796–802.
- de Wit, M., Kegge, W., Evers, J.B., Vergeer-van Eijk, M.H., Gankema, P., Voeseek, L.A.C.J. and Pierik, R. (2012) Plant neighbor detection through touching leaf tips precedes phytochrome signals. *Proc. Natl Acad. Sci. USA*, **109**(36), 14705–14710.
- de Wit, M., Galvão, V.C. and Fankhauser, C. (2016) Light-mediated hormonal regulation of plant growth and development. *Annu. Rev. Plant Biol.* **67**(1), 513–537.
- de Wit, M., Keuskamp, D.H., Bongers, F.J. et al. (2017) Integration of phytochrome and cryptochrome signals determines plant growth during competition for light. *Curr. Biol.* **26**(24), 3320–3326.
- Worthington, M. and Reberg-Horton, C. (2013) Breeding cereal crops for enhanced weed suppression: optimizing allelopathy and competitive ability. *J. Chem. Ecol.* **39**(2), 213–231.
- Wu, H., Pratley, J., Ma, W. and Haig, T. (2003) Quantitative trait loci and molecular markers associated with wheat allelopathy. *Theor. Appl. Genet.* **107**(8), 1477–1481.
- Xiao, J., Zhang, H., Xing, L., Xu, S., Liu, H., Chong, K. and Xu, Y. (2013) Requirement of histone acetyltransferases HAM1 and HAM2 for epigenetic modification of *FLC* in regulating flowering in *Arabidopsis*. *J. Plant Physiol.* **170**(4), 444–451.
- Xu, W., Liu, D., Wu, F. and Liu, S. (2015) Root exudates of wheat are involved in suppression of *Fusarium* wilt in watermelon in watermelon-wheat companion cropping. *Eur. J. Plant Pathol.* **141**(1), 209–216.
- Yang, L., Callaway, R.M. and Atwater, D.Z. (2017) Ecotypic diversity of a dominant grassland species resists exotic invasion. *Biol. Invasions*, **19**(5), 1483–1493.
- Zeng, D., Qian, Q., Teng, S., Dong, G., Fujimoto, H., Yasufumi, K. and Zhu, L. (2003) Genetic analysis of rice allelopathy. *Chin. Sci. Bull.* **48**(3), 265–268.
- Zhang, H., Kim, M.S., Krishnamachari, V. et al. (2007) Rhizobacterial volatile emissions regulate auxin homeostasis and cell expansion in *Arabidopsis*. *Planta*, **226**(4), 839–851.
- Zhang, D., Zhang, C., Tang, X., Li, H., Zhang, F., Rengel, Z., Whalley, W.R., Davies, W.J. and Shen, J. (2016) Increased soil phosphorus availability induced by faba bean root exudation stimulates root growth and phosphorus uptake in neighbouring maize. *New Phytol.* **209**, 823–831.
- Zuo, S., Liu, G. and Li, M. (2012) Genetic basis of allelopathic potential of winter wheat based on the perspective of quantitative trait locus. *Field. Crop. Res.* **135**, 67–73.

**I.C Towards unifying evolutionary ecology and genomics to understand genotype-by-genotype interactions within wild plant species** (Submitted to *Plant Communications*)

# **Towards unifying evolutionary ecology and genomics to understand genotype-by-genotype interactions within wild plant species**

Harihar Jaishree Subrahmaniam<sup>1</sup>, Dominique Roby<sup>1</sup> and Fabrice Roux<sup>1,\*</sup>

<sup>1</sup>LIPM, Université de Toulouse, INRA, CNRS, Castanet-Tolosan, France

\* To whom correspondence should be addressed. E-mail: [fabrice.roux@inra.fr](mailto:fabrice.roux@inra.fr)

**Keywords:** intraspecific variation, genotype-by-genotype interactions, kin cooperation, diversity-productivity relationship, genotype-by-genotype-by-environment interactions, over-yielding.

## **ABSTRACT**

In a local environment, plant social networks include interactions between individuals of different species and among genotypes of the same species. While interspecific interactions have been highly recognized as a main driver of plant community patterns, intraspecific interactions have recently gained attention in explaining plant community dynamics. However, an overview of intraspecific genotype-by-genotype interaction patterns within wild plant species is still missing. The 77 experiments that we identified were mainly designed to test for the presence of positive interactions. Both the kin selection theory and elbow-room hypothesis were highly supported, despite their opposite predictions between the extent of genetic relatedness among neighbors and the level of positive interactions. In addition, we found that kin cooperation and overyielding were dominant in annual and perennial species, respectively. Nonetheless, we identified several shortcomings regardless of species considered, such as the lack of a reliable estimate of genetic relatedness among genotypes and ecological characterization of the natural habitats from which genotypes have been collected, which in turn impedes the identification of selective drivers of positive interactions. We therefore propose a framework combining evolutionary ecology and genomics in order to establish the eco-genetic landscape of positive GxG interactions in wild plant species.

## INTRODUCTION

During the course of its life cycle, a plant can interact directly or indirectly - consecutively and/or concurrently - with multiple neighboring plants. Plant social networks include interactions between individuals of different species (i.e. interspecific interactions) and among genotypes of the same species (i.e. intraspecific interactions) in a local environment. Plant–plant interactions play an important role in regulating the diversity and structure of plant communities and ultimately ecosystems functioning through their effects on resource availability and habitat structure (Brooker 2006, Martorell & Freckleton 2014). Studying the mechanisms underlying plant-plant interactions is therefore essential to understand the dynamics of plant communities, which may in turn help to predict the resilience of plant species in presence of anthropogenic-related global changes (Subrahmaniam *et al.* 2018). For instance, ongoing climate warming results in modifications of plant assemblages due to increase of plant biomass, reduced diversity (Baldwin *et al.* 2014) and shifts in the distribution areas of plant species (Gilman *et al.* 2010, Singer *et al.* 2013).

Plant-plant interactions can be divided into four main categories depending on the net benefit and cost associated with the interaction (Subrahmaniam *et al.* 2018). First, competitive interactions (-/-) come with a cost for both partners (benefit  $< 0$ , cost  $> 0$  for both partners). Competition is characterized by reciprocal negative effects on plant growth or fitness caused by the presence of neighbors (Keddy 2015). Since all plants share a few basic requirements, limitations of resources such as the availability of nutrients, water or light could drive competition between plants (Turkington & Harper 1979, Chaney & Baucom 2014). Second, asymmetric interactions (+/-) yield benefit to one of the partner at the cost of the other interactor (benefit  $< 0$  and cost  $> 0$  for the helper; benefit  $> 0$  and cost  $< 0$  for the receiver). Parasitic plants are the prime example of this kind of behavior. In addition, plants releasing allelochemicals to negatively influence the physiology of their neighbors can be grouped under this category. Third, commensal interactions (+/0) are those that are beneficial for at least one of the partners, but there is no cost associated with providing such aid (benefit = 0 and cost = 0 for helper; benefit  $> 0$  and cost = 0 for the receiver of the help). Many examples of such interactions exist at the interspecific level, like nurse plant effects in deserts or climbing plants that use the stems of other plants to avoid shade (Padilla & Pugnaire 2006, Gianoli 2015). Fourth, individuals can also reciprocally benefit (+/+) from being associated with a partner (benefit  $> 0$  and cost  $< 0$  for both plant partners). Many

examples of such a reciprocal help have been described at the interspecific level. Plant-mycorrhizal associations that help nutrient sharing and transfer between different plant species are one example of such an association (Teste *et al.* 2014).

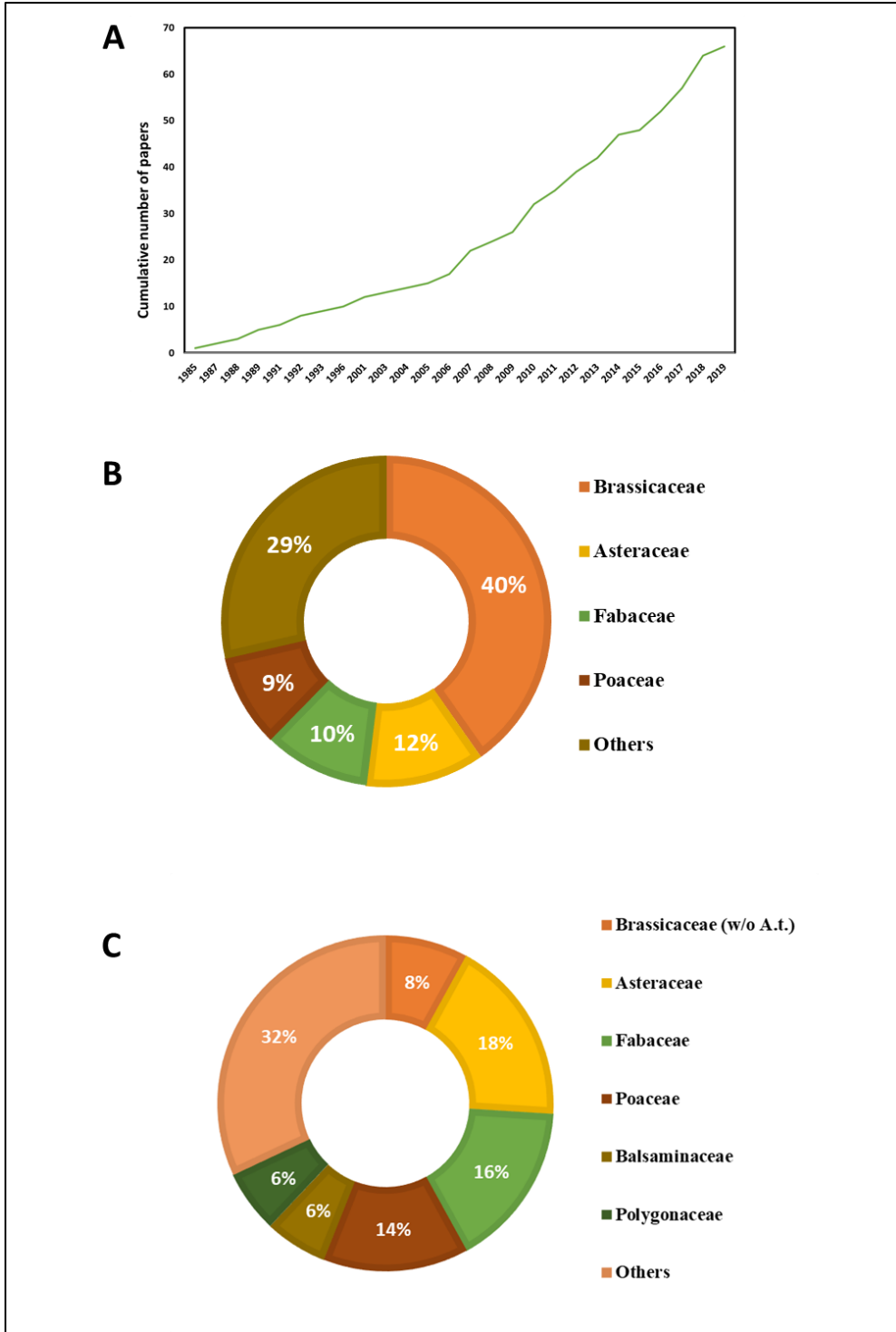
Estimating the relative importance of these broad categories in explaining patterns of plant communities is still under debate and mainly focused on interactions at the interspecific level. Interspecific competitive interactions have been traditionally recognized as the major factor responsible for the structure (Goldberg & Barton 1992), diversity (Chesson 2000) and dynamics of plant communities (Tilman 1985). However, more recently, the role of positive interactions among species (including both commensal interactions and reciprocal help) in regulating the composition of communities, has particularly gained attention. (Bertness & Callaway 1994, Callaway 1995, Brooker & Callaghan 1998, Bruno *et al.* 2003, Dormann & Brooker 2002, Kotowska *et al.* 2010, Wendling *et al.* 2017). In particular, positive interactions among species have been put forward to explain overyielding, which corresponds to the increase in productivity of species when grown in mixture as opposed to monoculture (Harper 1977, Vandermeer 1981, Loreau 2004, Schmid *et al.* 2008). However, upon decomposing species interactions into interactions occurring between genotypes of species, it has recently been argued that the interaction outcome depends on the genotype identity, rather than species identity (Ehlers *et al.* 2016). Genotype-by-genotype (GxG) interactions at the interspecific level might ultimately govern community diversity, composition and structure (Brooker 2006, Ehlers *et al.* 2016). Similarly, it is increasingly being recognized that studying GxG interactions at the intraspecific level might be a prerequisite for understanding eco-evolutionary patterns of plant communities (Hughes *et al.* 2008, Lankau 2018). Indeed, a huge number of genotypes of varying levels of relatedness can co-exist within a local population, even in the case of highly selfing species. For instance, a recent study on *Arabidopsis thaliana* revealed that the genetic diversity observed within a local population represents almost one-sixth of the genetic diversity at the worldwide scale (Frachon *et al.* 2017). Therefore, the patterns of interactions between different genotypes within one population are bound to vary as well.

Several meta-analyses have been carried out to understand patterns of GxG interactions at the interspecific level in herbaceous wild plant species (Maestre *et al.* 2005) as well as in trees (Piotto 2008, Zhang *et al.* 2012). However, an overview of GxG interaction patterns at the intraspecific

level within wild plant species is still missing from literature. This review therefore aims to make a synthesis on such interactions. More precisely, based on 66 articles, we aimed at establishing general patterns of intraspecific GxG interactions by addressing the following questions: (i) Why GxG interactions were studied for ?, (ii) What plant material was used to study GxG interactions ?, (iii) What were the growth conditions used to estimate GxG interactions ?, (iv) What traits were phenotyped to study GxG interactions ?, (v) Can interactions between genotypes be indirect ?, and (vi) What major conclusions can be reached in GxG experimental studies ? We then introduce several avenues that deserve to be explored to obtain a thorough picture of GxG interaction patterns within wild species. In particular, we stress the need to integrate genomics and evolutionary ecology to fully understand the complexity of intraspecific genetic interactions in wild plant populations.

## **SURVEY OF LITERATURE AND HIGHLIGHTS ON LIFE-HISTORY AND ECOLOGICAL FEATURES OF THE SPECIES**

For this review, we only focused on studies looking at intraspecific interactions within wild herbaceous species. We made this choice because the number of generations of wild herbaceous species is clearly smaller than the one of trees, therefore fitness proxies can be better estimated during their life cycle. Several keywords were used to gather these studies: GxG interactions, intraspecific interactions, intraspecific variation, intra/inter-population variation, group selection. The websites inspected included Google Scholar, Web of Science, Sci-hub, Researcher. Although we tried to do a comprehensive analysis to include a maximum number of studies reporting intraspecific GxG interactions, the list is certainly not exhaustive and some studies may have been gone overlooked. We gathered a list of 66 articles including 77 experiments (Supplementary Table 1) published in the last 35 years. Interestingly, we observed a sharp increase in cumulative number of experimental papers published over the years (Figure 1A), thereby illustrating the rising interest in examining intraspecific GxG interactions in wild plant species. The list includes 43 species belonging to 18 botanical families (Figure 1B). The most commonly studied botanical families comprise of Brassicaceae (40%), Asteraceae (12%), Fabaceae (10%) and Poaceae (9%). However, there is a significant bias in the family Brassicaceae towards *A. thaliana* as it constitutes about



**Figure 1: Surveying GxG interactions within herbaceous wild species.** A. Cumulative number of papers on GxG interactions in wild plant species from 1985 to 2019. B. Doughnut plots describing the distribution of the major botanical families used in the study of intraspecific GxG interactions. C. Doughnut plots describing the distribution of the major botanical families used in the study of intraspecific GxG interactions without including *A. thaliana*.

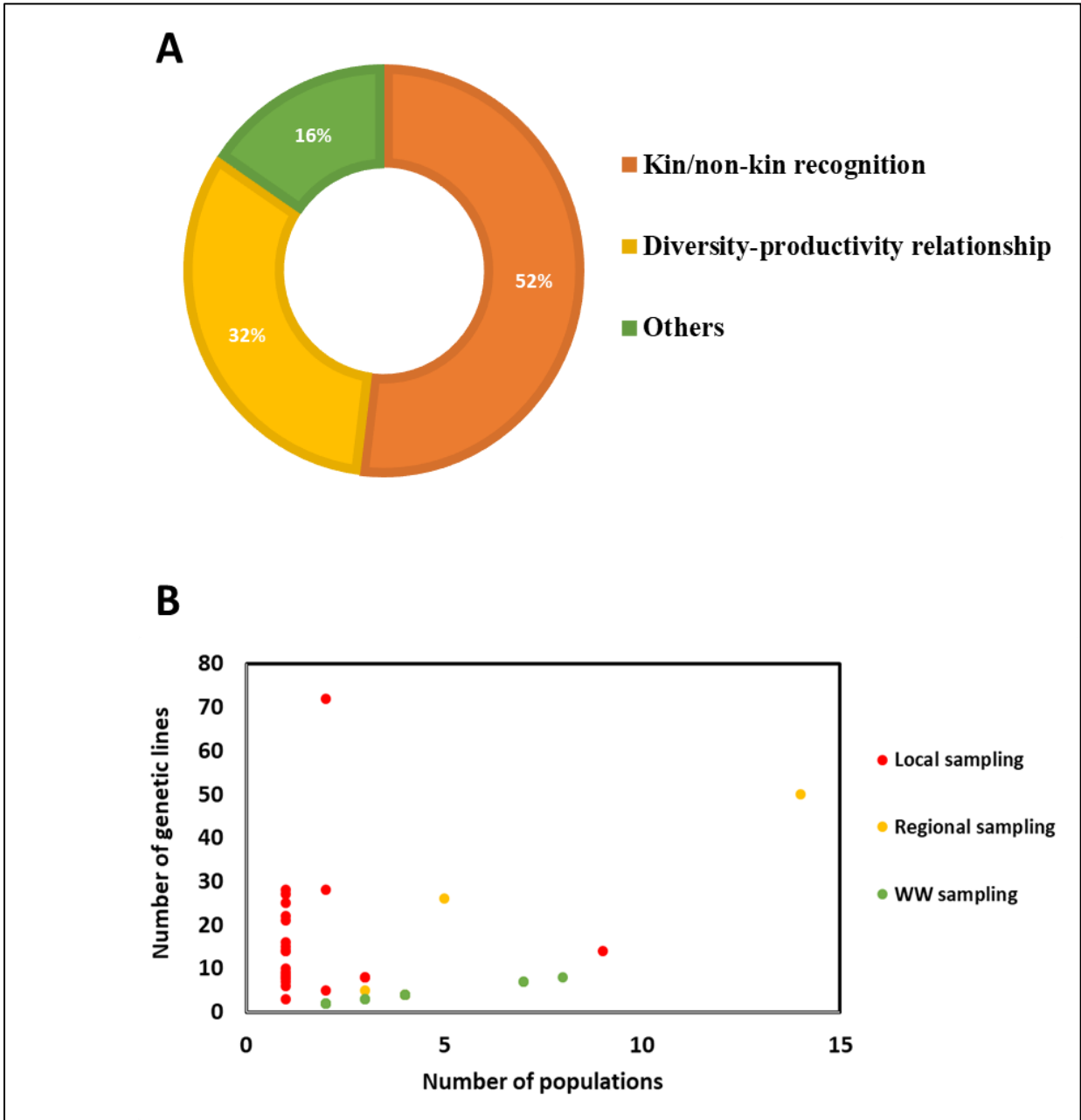
90% of the studies from this family. Upon removing this species, the relative proportion of botanical families studied is consistent with the amount of species within each family (Figure 1B, Supplementary Table 1). The list of 43 species is divided roughly equally between annuals (~46%) and perennials (~54%) (Supplementary Table 1) and is dominated by selfing species that make up about 42% of the dataset. The remaining species comprise of mixed breeding system (28%), outcrossing (21%) and clonals (9%). Allochory (assisted seed dispersal) seems to be predominant in this list as about 65% of the listed species demonstrated this mode of seed dispersal, while only 35% of species listed have an autochorous (self) mode of seed dispersal (Supplementary Table 1).

## **GENERAL PATTERNS OF GxG INTERACTIONS AT THE INTRASPECIFIC LEVEL**

### ***Why GxG interactions were studied for?***

The reported experiments can be categorized into two main rationales that hypothesize opposite relationships between the extent of genetic relatedness among neighbors and the level of positive interactions (File *et al.* 2012). Rooted in evolutionary biology concepts, the first rationale is based on the kin selection theory that advocates that individuals increase their inclusive fitness by modifying their behavior to help a relative (Hamilton 1964). The ‘kin/non-kin recognition’ category concerns 52% of the experiments where the differential response of a genotype was tested in the presence/absence of a relative genotype (kin) *vs* a stranger genotype in pairwise experiments (Figure 2A, Supplementary Table 1). The second rationale is based on the elbow-room ecological hypothesis that assumes that intraspecific resource partitioning occurs and increases as the genetic distance between neighbors increases (Argyres & Schmitt 1992). This positive diversity-productivity relationship corresponds to overyielding at the intraspecific level. The ‘genotypic diversity- productivity relationship’ category concerns 32% of the experiments where fitness proxies were compared between monocultures using multiple kin individuals (compound intra-genotypic interactions) and mixtures of different genotypes (compound inter-genotypic interactions) (Figure 2A, Supplementary Table 1).

The remaining experiments (16%) that were grouped under the category ‘Others’ included experiments that aimed at (i) characterizing the genetic architecture underlying GxG interactions



**Figure 2: Classification of experimental studies on GxG interactions.** A. Doughnut plot describing the distribution of the papers according to the rationale of studying GxG interactions. B. Scatterplot depicting biological samples and geographical scale of sampling used in the different experimental studies. WW: worldwide. NB: for better illustration, the outlier with 60 genotypes each from a different population is not incorporated in the figure (Fitzpatrick *et al.* 2019).

(Mutic & Wolf 2007, Botto & Callucio 2007), (ii) studying the effects of GxG interactions on intra-individual traits such as genome size variation (Smarda *et al.* 2010) and transcriptomic profiles (Bowsher *et al.* 2007), (iii) studying extended phenotypes such as root exudate profiles (Badri *et al.* 2012) and soil microbial communities (Burghardt *et al.* 2019, Fitzpatrick *et al.* 2019), (iv) testing the effect of adding a neighbor plant on genotype-by-environment interactions (i.e. GxExG instead of GxGxE) (Cahill *et al.* 2010), (v) looking at local adaptation of genotypes (Linhart 1988, Espeland & Rice 2007), and (vi) investigating individual *vs* group selection in wild plant populations (Goodnight *et al.* 1985, Donohue 2003). For the latter, we need to stress that the existence of group selection is still controversial (Nowak 2006, Nowak *et al.* 2010, Rousset & Lion 2011, Queller *et al.* 2015, Kramer & Meunier 2016) and it will not be addressed in this review.

### ***What plant material was used to study GxG interactions?***

Around 84% of the experiments listed were based on genotypes collected in natural populations (Supplementary Table 1). With the main goal of dissecting the underlying genetic and molecular mechanisms of GxG interactions, the remaining experiments were based on (i) experimental populations such as F<sub>2</sub> populations or families of Recombinant Inbred Lines (RILS, 9%) (Goodnight *et al.* 1985, Griffing 1989, Mutic & Wolf 2007, Botto & Collucio 2007, Willis *et al.* 2010, Latzel *et al.* 2013, Wuest & Niklaus 2018), and (ii) mutant lines (~5%) (Cahill *et al.* 2005, Crepy & Casal 2014, Wagg *et al.* 2014, Zhang & Tielborger 2018) (Supplementary Table 1). Unsurprisingly, given the great amount of genetic resources publicly available, all these experiments dedicated to the study of genetic and molecular mechanisms concern *A.thaliana* (with the exception of *Trifolium pratense*, Wagg *et al.* 2014).

For experiments based on genotypes collected in natural populations, we observed a clear tradeoff between the number of genotypes used and the number of populations sampled (Figure 2B). The number of natural genetic lines used to evaluate GxG interactions is highly variable among experiments, ranging from 2 to 72 (mean ~12) (Figure 2B). On average, these lines have been collected from about four populations (min=1, max=60) (Figure 2B). In wild plant populations, intraspecific neighbors share common space over generations and this increases their probability for repeated interactions. Consequently, positive interactions are also likely to evolve between members of a single population rather than between members of different populations

(Nowak 2006). Accordingly, most experiments that do not imply *A. thaliana* are based on natural genetic lines from a single population (30%) or sampled at a regional scale (between 2-14 populations, 37.6%). On the other hand, an opposite trend is observed in *A. thaliana*, which represents 35% of the dataset. Almost 90% of the experiments on this species utilized genotypes coming from worldwide collections. The main hypothesis to explain this bias in using worldwide genotypes in *A. thaliana* is related to its predominantly selfing breeding system, which initially suggested that most populations were monomorphic (Platt *et al.* 2010). Therefore, the large public collections of genotypes that are available for *A. thaliana* mostly correspond to one representative genotype per population. However, more and more studies challenged this view by revealing extensive genetic diversity within populations (Le Corre 2005, Jorgensen & Emerson 2008, Bomblies *et al.* 2010, Platt *et al.* 2010, Kronholm *et al.* 2012, Brachi *et al.* 2012, Roux & Bergelson 2016, Frachon *et al.* 2017, Fulgione *et al.* 2017, Frachon *et al.* 2018), thereby giving an opportunity of studying more relevant GxG interactions in *A. thaliana* at the local scale.

Based on all the experimental experiments listed in this survey, we nonetheless identified two major shortcomings of the plant material used to study GxG interactions, regardless of species considered. Firstly, as previously mentioned, testing both the kin selection and elbow-room hypotheses requests estimation of the degree of genetic relatedness among interacting genotypes. Kin selection theory predicts partisan help given to close relatives. By contrast, according to the elbow-room hypothesis, genetically close relatives will compete for the same resources and increasing genetic distance between genotypes can translate into increasing niche partitioning. To test for these contrasting predictions would require integrating information about the extent of genetic relatedness among interacting genotypes. However, this crucial information has been poorly considered in these experiments. In our survey, only two experiments estimated the degree of genetic relatedness among interacting genotypes (Crutsinger *et al.* 2006, Crutsinger *et al.* 2008) (Supplementary Table 1). Secondly, positive plant-plant interactions at the interspecific level are expected to evolve in natural settings including a certain level of abiotic and/or biotic stress (Bertness & Callaway 1994, Brooker & Callaghan 1998, Bruno *et al.* 2003). Whether this stress gradient hypothesis is also relevant at the intraspecific level remains an open question. Still, in our survey, only 19% of the experiments have loosely described the ecology of the populations used in the experiments (Supplementary Table 1). At most, only a rough description of habitats from which the genotypes have been collected was given.

### ***What were the growth conditions used to estimate GxG interactions?***

Performing experiments in controlled and field conditions is complementary (Bergelson & Roux 2010, Brachi *et al.* 2010). Experiments conducted under controlled conditions drastically reduce environmental noise, thereby allowing establishing a direct link between phenotypic observations and genotype performance under a given set of stable environmental conditions. On the other hand, in the field, plants are exposed to a greater but more ecologically realistic range of abiotic and biotic fluctuations than typically encountered in controlled conditions. Nonetheless, encompassing all these environmental fluctuations request the field experiments to be repeated over several years.

In our survey, almost 79% of the experiments were conducted under laboratory conditions (Supplementary Table 1). Out of these, ~67% and ~21% of the experiments were conducted in greenhouse conditions and growth chambers (including root chambers and growth tunnels), respectively. The remaining experiments (~12%) have been performed under *in vitro* conditions. On the other hand, few experiments (~13%) have been conducted under field conditions, even less in the native habitats (only two reported experiments, Supplementary Table 1). Finally, four experiments (~5%) were conducted in both greenhouse and field conditions (Espeland & Rice 2007, Anderson 2014, Ehlers *et al.* 2016). The type of growth conditions used to study intraspecific GxG interactions is therefore strongly biased in favor of laboratory conditions, notably when compared to other types of biotic interactions. For instance, in a recent review on Genome-Wide Association studies (GWAS) performed on plant – pathogen interactions, 60% of the studies were conducted in controlled conditions (greenhouse/growth chambers) and 40% under field conditions (Bartoli & Roux 2017).

Noteworthy, around 66% of the experiments in our survey tested the effect of a particular environmental factor on GxG interactions, either in controlled or field conditions (Supplementary Table 1). Abiotic treatments concern light quality, nutrient status, CO<sub>2</sub> concentration and drought, whereas biotic treatments mainly concern density and the effect of soil conditioning by one or a mixture of genotypes, in particular for the ‘diversity-productivity relationship’ rationale (Bukowski & Petermann 2014, Semchenko *et al.* 2017, Bukowski *et al.* 2018). However, since no

thorough ecological characterization has been conducted on the habitats from which the plant material has been collected, the treatments applied may not be ecologically relevant.

### ***What traits were phenotyped to study GxG interactions?***

All the traits measured across the 77 experiments are listed in Supplementary Table 2. To assess GxG interactions, an average of 3.4 traits per study have been measured (min=1, max=9). We divided the list of traits into four broad categories, each related to a distinct eco-function of the plant (i.e. root related traits, shoot related traits, life history traits and seed production related traits). While ~43% of the experiments scored life history related traits (e.g. germination and flowering timing), ~53% and ~79% of the experiments measured root (e.g. root length and biomass) and shoot (e.g. plant height and dry biomass) related traits, respectively. Seed production related traits (e.g. number of fruits and number of seeds per fruit) were measured in ~40% of the experiments. Interestingly, most experiments focused on collecting phenotypic information using either two (45%) or three (27.2%) categories. About 39% of the experiments looked at both root and shoot related phenotype while ~34% of experiments focused on both shoot related and life history related traits. Only four experiments (~5%) focused on all four categories (Wilson *et al.* 1987, Linhart *et al.* 1988, Masclaux *et al.* 2009). The relative proportion of trait categories are similar between the different rationales (Supp. Fig 1).

Measuring individual specific root responses is often recognized to be very difficult in experiments on plant-plant interactions. More often than not, to measure specific root traits, the total root biomass contributed by all genotypes present in a pot has been considered as a dependable measure, which obviously impedes the estimation of the relative contribution of each individual genotype in the pot. However, developments in non-destructive phenotyping technology has added new directions to start teasing apart the respective underground behavior of each genotype in both laboratory and field/natural experimental setups. For example, the 3D root system architecture of plants within natural or field soils can be easily created using low invasive tools (minirhizotrons; Johnson *et al.* 2001). More recently, X-ray Computed Tomography has been described to be useful for studying in details the root system, such as lateral root growth and orientation patterns under laboratory conditions (Subramanian *et al.* 2015).

Plant neighbor interactions are often dynamic in nature and can switch from competitive to positive interactions depending on (i) the environmental stress perceived (Bertness & Callaway 1992), (ii) life stages of interacting species (Walker & Vitousek 1991, Kellman & Kading 1992, Chapin *et al.* 1994, Pugnaire *et al.* 1996), or (iii) the physiological capacity of the interacting species (e.g. facilitation by improving plant water uptake under canopy shade or soil oxygenation; Callaway *et al.* 1996, Holmgren *et al.* 1997). In all the experiments listed in our survey, this potential switch of interactions has been overlooked. Testing for such complex dynamic GxG interactions would require taking phenotypic measurements from various plant compartments at multiple time points.

### ***Can interactions between genotypes be indirect?***

We further categorized the experiments depending on whether the interactions tested were direct or indirect. We classified the interactions to be indirect when the treatment did not include placing at least two genotypes in a pot together. In our survey, such indirect interactions have been addressed in ~6% of the 77 experimental studies (Supplementary Table 1). Indirect interactions between genotypes can occur by two main processes. Firstly, soil conditioning can mediate indirect interactions between genotypes. In our survey, all but one studies listed on indirect interactions tested the effect of soil conditioning by one genotype for its effect on the growth response of another genotype. More precisely, such experiments were conducted to test for the effect of soil microbiota in driving GxG interactions (Aguilera *et al.* 2011, Wagg *et al.* 2014). Soil biota mediated plant soil feedback (PSF) has been abundantly documented at interspecific level, and has been recognized in driving ecosystem processes by multiple studies (Klironomos 2002, Kardol *et al.* 2006, 2007, Reinhart & Callaway 2006, Petermann *et al.* 2008). Negative PSF has also been demonstrated at intraspecific level (Wagg *et al.* 2014), and has been indicated to have a role in maintaining intraspecific coexistence (Bukowski *et al.* 2018). The composition of root exudates, which is genotype specific, defines the assembly of distinct soil microbial communities in the rhizosphere. Consequently, different genotypes in one population can recruit different microbiota. These microbiota may have a central role in mediating GxG interactions in natural plant communities and their coexistence within one population. Similarly, the role of microbiota in influencing social behavior of vertebrates has been experimentally demonstrated (Klein *et al.* 2003), which is in line with recent theoretical demonstration describing the putative role of

microbes on explaining altruism between organisms (Lewin-Epstein *et al.* 2017). Yet, the genuine role of PSF in shaping evolution of plant populations is largely unexplored (Wagg 2015), and this scarcity of studies looking at the role of microbiota on GxG interactions represents a future challenge.

Secondly, indirect interactions between genotypes can be mediated by volatile organic compounds (VOCs) both aboveground and underground. Plant produced VOCs helping in neighbor detection (Ninkovic *et al.* 2019) is known to affect intraspecific competition in crops (Ninkovic 2003) and trees (Ormeno *et al.* 2007). In our survey, only one experiment focused on studying indirect interactions through aerial VOCs in *Artemisia tridentata* (Karban *et al.* 2013). The cocktail of VOCs has been found to be highly variable among individuals and has been discussed to help distinguishing kin and stranger genotypes (Karban *et al.* 2014). Still, there is a caveat in understanding the exact role of VOCs in indirect GxG interactions in wild species and how they can affect intraspecific communication between genotypes in natural conditions.

### ***Outcomes of GxG studies: justifying major rationales?***

We divided the major conclusions from the 77 listed experiments into (i) *kin cooperation* (KC) for studies demonstrating positive interactions between kins, (ii) *overyielding* (OY) for studies where the mixture of genotypes was confirmed to be more productive than monocultures, and (iii) *neutral* for studies where no significant differences were observed between the treatments specified in the experiments. While only ~21% studies found no significant differences between treatments, ~46% reported KC and ~31% studies reported OY, (Supplementary Table 1). The relative proportion of KC, OY and neutral interactions in the survey can be matched to the two main rationales used in the experimental studies. Studies testing for kin/non-kin recognition confirmed more KC, while studies aiming at testing diversity-productivity relationships, corroborated more cases of OY (Figure 3). However, we need to stress that the amount of studies reporting no differences could be more than what is listed in this survey, owing to the tendency of not publishing non-significant results.

When considering all the experiments, the relative proportion of KC, OY or neutral was not dependent on the annuality *vs* perenniality life history strategy ( $\chi^2 = 2.4$ ,  $P = 0.11$ ; Figure 4), the breeding system (selfing & clonal *vs* outcrossing & mixed,  $\chi^2 = 0.14$ ,  $P = 0.7$ ; Supplementary Figure 2), the mode of seed dispersal (autochory *vs* allochory,  $\chi^2 = 0.80$ ,  $P = 0.6$ ; Supplementary

Figure 3) and the geographical scale of sampling (one population vs multiple populations,  $\chi^2 = 0.68$ ,  $P = 0.4$ ; Supplementary Figure 4). Similar results were obtained when removing *A. thaliana* from the list of species (Supplementary Figures 2, 3 and 4), with the exception of the annuality vs perenniality life history strategy ( $\chi^2 = 5.52$ ,  $P = 0.01$ ; Figure 4). KC was detected in 13 out of the 16 experiments (~80%) conducted on annual species. On the other hand, KC was detected in only 5 out of the 20 experiments (~25%) conducted on perennial species, OY being detected in most of the remaining studies. Taking into account that predominantly self-pollinating plant species have an annual life history (Snell & Aarsen 2005), an explanation for these observations might be linked to the presence of neighbors of high relatedness (owing to selfing) in natural populations. The highest proportion of OY observed in perennials could be that, in a perennial life cycle, co-existing kin genotypes would possibly spend more time utilizing and competing for the same resources. In addition, perennial life cycle offers ample chances for a genotype to interact with multiple intraspecific neighbors. This may allow the creation of cooperative links with neighbors of varying levels of relatedness, thereby favoring overyielding in mixtures.

At the interspecific level, increase in magnitude of positive interactions has been linked to varying levels of abiotic (Maestre *et al.* 2009) and biotic stresses (Smit *et al.* 2009) in various studies. In our survey, in line with the equivocal results for individual performances in presence of relative (kin) vs stranger genotypes obtained for wild plant species (File *et al.* 2012), we identified two studies reporting a shift from KC to OY occurring between the same genotypes in response to a particular environmental factor. Firstly, in *A. thaliana*, genotypes performed better with kins under ambient CO<sub>2</sub> concentrations, while genotypic mixtures were favoured under elevated CO<sub>2</sub> concentrations (Andalo *et al.* 2001). Secondly, in *Phalaris arundinacea*, genotypes performed better with kins in disturbed plots (with all native vegetation removed), while genotypic mixtures were confirmed to perform better in undisturbed plots (Collins *et al.* 2018). Both experiments provide evidence of the existence of similar dynamics of intraspecific positive interactions among different species by varying environments.

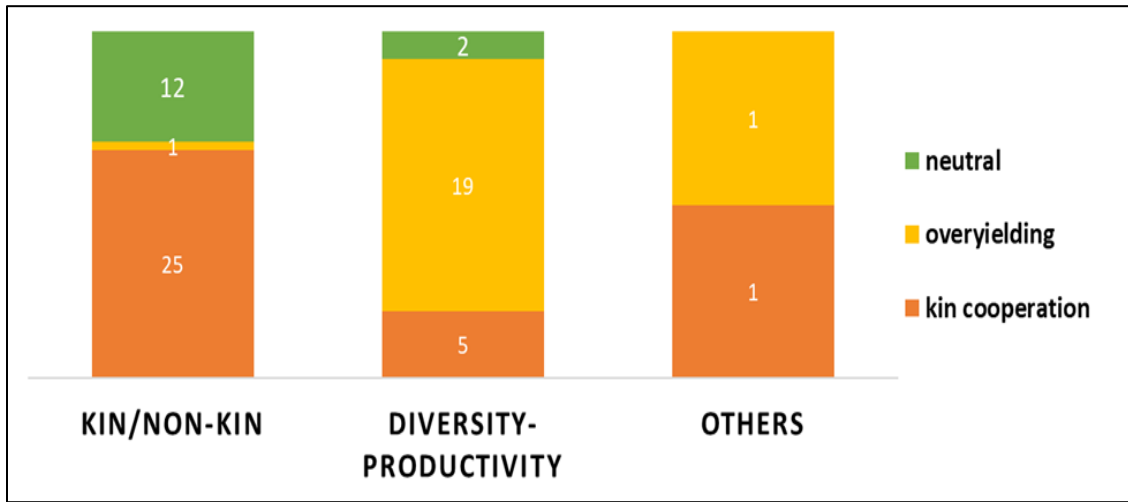


Figure 3: Stacked barplots illustrating the relative proportion of kin cooperation (KC), overyielding (OY) and neutral interactions observed between different rationales.

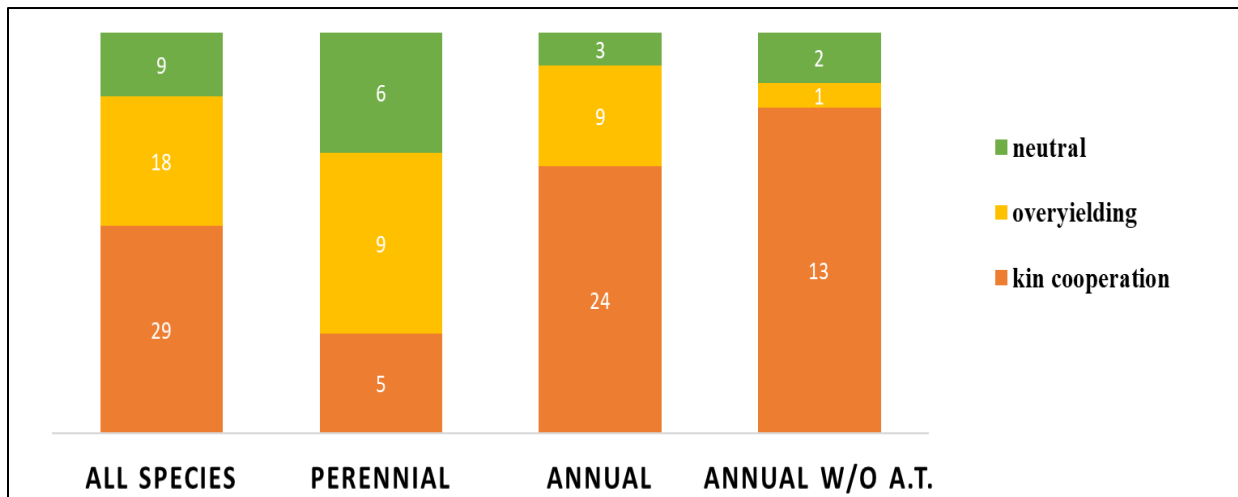
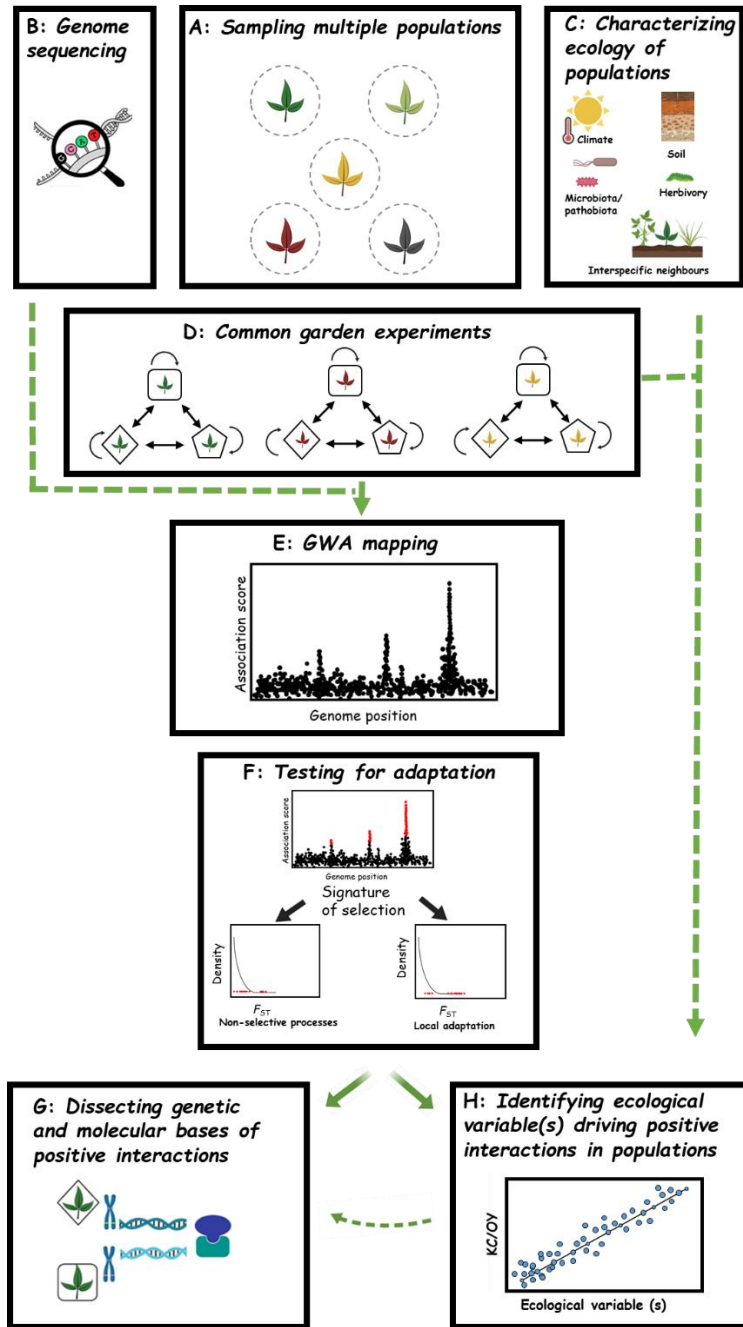


Figure 4: Stacked barplots illustrating the different outcomes of GxG interactions based on the annuality vs perennality strategy of species studied (with and without including *Arabidopsis thaliana*).

## FUTURE AVENUES

Despite the demonstration of positive interactions in 27 species, the underlying genetic architecture is still an open question. In our survey, we identified only one study that addressed this challenge. By using 37 RILs, a single major QTL was detected as underlying kin cooperation in *A. thaliana* (Wuest & Niklaus 2018). While informative, the low number of RILs used precluded a proper characterization of the genetic architecture (Keurentjes *et al.* 2007). In addition, it remains (i) to be tested whether polymorphic genes involved in positive interactions have been shaped by natural selection and (ii) to identify the ecological factors driving adaptive KC or OY (Subrahmaniam *et al.* 2018, Frachon *et al.* 2019). Here, we therefore introduce a framework in order to establish a genomic map of local adaptive positive interactions (Figure 5).

The first step would be collecting genetic material from multiple local populations (Figure 5A). In order to reduce the rate of false positives due to population structure during Genome-Wide Association (GWA) mapping (see below), the sampling should be limited to populations that experienced the same demographic history, as previously advocated in studying the co-evolutionary genetics of plant-pathogen interactions (Bartoli & Roux 2017). Then, the genomic characterization of the plant material will not only allow estimating the genetic relatedness among genotypes within and between local populations, but will also be essential to run GWA mapping (see below, Figure 5B). Simultaneously, an extensive *in situ* characterization of both abiotic and biotic ecological factors should be conducted for each population (Figure 5C). This is a crucial step to identify the main stresses experienced by the species, which may in turn help to simulate ecologically realistic treatments in phenotyping experiments and identify the selective drivers acting on positive interactions. While abiotic stress characterization is facilitated by publicly available databases for climate and dedicated platforms for soil properties, characterizing biotic factors can still be strenuous and time consuming. However, with the ever-decreasing cost of next-generation sequencing (NGS) technologies, describing the taxonomic and functional social networks (microbes, plants, insects...) of hundreds of wild plant populations is not out of reach (Bartoli *et al.* 2018, Frachon *et al.* 2019).



**Figure 5: Future avenues for studying GxG interactions among wild plant populations.** A: Collecting multiple genotypes in several natural populations. B: Genome sequencing of all genotypes coming from different populations. C: Characterizing abiotic and biotic ecological factors. D: Conducting common garden experiments. Dissecting pairwise interactions among genotypes from one population (intra-genotype and intergenotype intra-population combinations). Genotypes are indicated by using different shapes. E: Genome Wide Association mapping to identify genomic regions underlying GxG interactions, e.g. positive interactions (KC: Kin cooperation, OY: Overyielding). F: Identifying putative ecological driver(s) associated with among-population variation of GxG interactions, e.g. positive interactions. G: Enrichment analysis in signatures of selection, e.g. spatial genetic differentiation estimated by  $F_{ST}$ . H: Functional characterization of genes underlying QTLs associated with positive interactions.

In order to estimate the natural variation of GxG interaction patterns among populations, the next step would be to conduct common garden experiments by estimating differences at the intra-population level between intra-genotypic interactions (monoculture) and inter-genotype pairwise interactions (Figure 5D). Although the best strategy would be to perform experiments in the native habitats from which the populations have been collected, this approach may not be manageable for large number of populations. Alternatively, we recommend setting up phenotyping experiments both in field and in controlled conditions, each allowing the application of treatments that would match the ecologically relevant stresses identified in the local populations. In addition, in order to obtain a full picture of the playful dynamics of traits involved in positive interactions, we advocate the need to measure multiple phenotypic traits representative of plant condition (i.e. traits related to leaves, roots, life history, seed production...) and/or a given phenotypic trait at multiple time points.

The next step would be to adopt a GWA mapping approach to fine map genomic regions associated with natural variation of positive interactions among populations (Figure 5E). GWA mapping approach has previously proved to be a powerful approach to identify QTLs associated with plant-plant interactions in many crop species (Subrahmaniam *et al.* 2018), and also with intraspecific intragenotype interactions and interspecific interactions in *A. thaliana* (Baron *et al.* 2015, Frachon *et al.* 2017). After this, the type and strength of selection acting on QTLs can be addressed by testing whether these QTLs are enriched in genomic regions with signatures of selection (e.g. adaptive spatial differentiation, adaptive temporal differentiation and 'hard' selective sweeps; Fournier-Level *et al.* 2011, Hancock *et al.* 2011, Horton *et al.* 2012, Brachi *et al.* 2015, Frachon *et al.* 2017) (Figure 5F).

After confirming the adaptive status of the genetic basis associated with natural variation of positive interactions, two complementary approaches can be adopted to understand the genetic and molecular mechanisms in an ecologically realistic context (Figures 5G and 5H). On one hand, QTL cloning can be achieved by characterization of mutants (T-DNA, EMS, etc) or transgenic lines with gain or loss of functions of the gene(s) of interest. Alternatively, CRISPR/Cas9 system can also be utilized to create gene knockouts. Furthermore, complementation with natural alleles can then reveal potential Quantitative Trait Polymorphisms such as nucleotide substitution, indels and local genomic rearrangements. On the other hand, the identification of the ecological factors

driving adaptive KC or OY and their underlying genetic basis, can be achieved by adopting bilinear factor models (such as sparse Partial Least Square Regression). Such models would allow identifying linear combinations of abiotic and biotic factors (see Figure 5C) maximizing the variance in KC or OY observed among populations (Figure 5H). This may in turn help to identify the growth conditions in which to clone QTLs.

Altogether, combining ecology and evolutionary biology, along with quantitative genetics, genomics and molecular biology represents an unprecedented opportunity to establish the genetic and molecular landscape of positive GxG interactions in wild plant species in their natural ecological landscape.

## **ACKNOWLEDGMENTS**

This work was funded by a PhD fellowship from the LABEX TULIP to HJS (ANR-10-LABX-41, ANR-11-IDEX-0002-02). The authors declare no conflict of interest

## References

- Abd El-Gawad, A. M., Zotti, M., Sarker, T. C., Mazzoleni, S., & Bonanomi, G. (2017). Does a plant detect its neighbor if it is kin or stranger? Evidence from a common garden experiment. *Community Ecology*, 18(3), 305–310. <https://doi.org/10.1556/168.2017.18.3.9>
- Abdala-Roberts, L., Pratt, R., Pratt, J. D., & Mooney, K. A. (2017). Traits underlying community consequences of plant intra-specific diversity. *PLoS ONE*, 12(9), 1–18. <https://doi.org/10.1371/journal.pone.0183493>
- Aguilera, A. G., Colón-Carmona, A., Kesseli, R., & Dukes, J. S. (2011). No accession-specific effect of rhizosphere soil communities on the growth and competition of *Arabidopsis thaliana* accessions. *PLoS ONE*, 6(11). <https://doi.org/10.1371/journal.pone.0027585>
- Andalo, C., Goldringer, I., & Godelle, B. (2001). Inter- and intragenotypic competition under elevated carbon dioxide in *Arabidopsis thaliana*. *Ecology*, 82(1), 157–164. <https://doi.org/10.2307/2680093>
- Andersson, S. (2014). Indirect genetic effects from competition in the clonal herb *Sedum album* (Crassulaceae). *PLoS ONE*, 9(8). <https://doi.org/10.1371/journal.pone.0106104>
- Argyres, A. Z., & Schmitt, J. (1992). Neighbor relatedness and competitive performance in *Impatiens capensis* (Balsaminaceae): a test of the resource partitioning hypothesis. *American Journal of Botany*, 79(2), 181–185. <https://doi.org/10.2307/2445106>
- Badri, D. V., de-la-Peña, C., Lei, Z., Manter, D. K., Chaparro, J. M., Guimarães, R. L., Summer, L.W., Vivanco, J. M. (2012). Root secreted metabolites and proteins are involved in the early events of plant-plant recognition prior to competition. *PLoS ONE*, 7(10). <https://doi.org/10.1371/journal.pone.0046640>
- Baker, B. H., Sultan, S. E., Lopez-Ichikawa, M., & Waterman, R. (2019). Transgenerational effects of parental light environment on progeny competitive performance and lifetime fitness. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 374(1768). <https://doi.org/10.1098/rstb.2018.0182>
- Baldwin, A. H., Jensen, K., & Schönfeldt, M. (2014). Warming increases plant biomass and reduces diversity across continents, latitudes, and species migration scenarios in experimental wetland communities. *Global Change Biology*, 20(3), 835–850. <https://doi.org/10.1111/gcb.12378>

- Baron, E., Richirt, J., Villoutreix, R., Amsellem, L., & Roux, F. (2015). The genetics of intra- and interspecific competitive response and effect in a local population of an annual plant species. *Functional Ecology*, 29(10), 1361–1370. <https://doi.org/10.1111/1365-2435.12436>
- Bartoli, C., Frachon, L., Barret, M., Rigal, M., Huard-Chauveau, C., Mayjonade, B., Zanchetta, C., Bouchez, O., Roby, D., Carrere, S., Roux, F. (2018). *In situ* relationships between microbiota and potential pathobiota in *Arabidopsis thaliana*. *ISME Journal*, 12(8), 2024–2038. <https://doi.org/10.1038/s41396-018-0152-7>
- Bartoli, C., & Roux, F. (2017). Genome-wide association studies in plant pathosystems: Toward an ecological genomics approach. *Frontiers in Plant Science*, 8(May). <https://doi.org/10.3389/fpls.2017.00763>
- Bergelson, J., & Roux, F. (2010). Towards identifying genes underlying ecologically relevant traits in *Arabidopsis thaliana*. *Nature Reviews Genetics*, 11(12), 867–879. <https://doi.org/10.1038/nrg2896>
- Bertness, M. D., & Callaway, R. (1994). Positive interactions in communities. *Trends in Ecology and Evolution*, 9(5), 187–191. [https://doi.org/10.1016/0169-5347\(94\)90087-6](https://doi.org/10.1016/0169-5347(94)90087-6)
- Bhatt, M. V., Khandelwal, A., & Dudley, S. A. (2011). Kin recognition, not competitive interactions, predicts root allocation in young *Cakile edentula* seedling pairs. *New Phytologist*, 189(4), 1135–1142. <https://doi.org/10.1111/j.1469-8137.2010.03548.x>
- Biedrzycki, M. L., & Bais, H. P. (2010). Kin recognition in plants: A mysterious behaviour unsolved. *Journal of Experimental Botany*, 61(15), 4123–4128. <https://doi.org/10.1093/jxb/erq250>
- Biedrzycki, M. L., Jilany, T. A., Dudley, S. A., & Bais, H. P. (2010). Root exudates mediate kin recognition in plants. *Communicative & Integrative Biology*, 3(1), 28–35. <https://doi.org/10.4161/cib.3.1.10118>
- Biedrzycki, M. L., L, V., & Bais, H. P. (2011). The role of ABC transporters in kin recognition in *Arabidopsis thaliana*. *Plant Signaling & Behavior*, 6(8), 1154–1161. <https://doi.org/10.4161/psb.6.8.15907>
- Biernaskie, J. M. (2011). Evidence for competition and cooperation among climbing plants. *Proceedings of the Royal Society B: Biological Sciences*, 278(1714), 1989–1996. <https://doi.org/10.1098/rspb.2010.1771>

- Bolin, L. G., Benning, J. W., & Moeller, D. A. (2018). Mycorrhizal interactions do not influence plant–herbivore interactions in populations of *Clarkia xantiana* ssp. *xantiana* spanning from center to margin of the geographic range. *Ecology and Evolution*, 8(22), 10743–10753. <https://doi.org/10.1002/ece3.4523>
- Bombliès, K., Yant, L., Laitinen, R. A., Kim, S. T., Hollister, J. D., Warthmann, N., Fitz, J., Weigel, D. (2010). Local-scale patterns of genetic variability, outcrossing, and spatial structure in natural stands of *Arabidopsis thaliana*. *PLoS Genetics*, 6(3), e1000890. doi:10.1371/journal.pgen.1000890
- Botto, J. F., & Coluccio, M. P. (2007). Seasonal and plant-density dependency for quantitative trait loci affecting flowering time in multiple populations of *Arabidopsis thaliana*. *Plant, Cell and Environment*, 30(11), 1465–1479. <https://doi.org/10.1111/j.1365-3040.2007.01722.x>
- Bowsher, A. W., Shetty, P., Anacker, B. L., Siefert, A., Strauss, S. Y., & Friesen, M. L. (2017). Transcriptomic responses to conspecific and congeneric competition in co-occurring *Trifolium*. *Journal of Ecology*, 105(3), 602–615. <https://doi.org/10.1111/1365-2745.12761>
- Brachi, B., Aimé, C., Glorieux, C., Cuguen, J., & Roux, F. (2012). Adaptive value of phenological traits in stressful environments: Predictions based on seed production and laboratory natural selection. *PLoS ONE*, 7(3), e32069. <https://doi.org/10.1371/journal.pone.0032069>
- Brachi, B., Faure, N., Horton, M., Flahauw, E., Vazquez, A., Nordborg, Bergelson, J., Cuguen, J., M., Roux, F. (2010). Linkage and association mapping of *Arabidopsis thaliana* flowering time in nature. *PLoS Genetics*, 6(5), 40. <https://doi.org/10.1371/journal.pgen.1000940>
- Brachi, B., Meyer, C. G., Villoutreix, R., Platt, A., Morton, T. C., Roux, F., & Bergelson, J. (2015). Co-selected genes determine adaptive variation in herbivore resistance throughout the native range of *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the United States of America*, 112(13), 4032–4037. <https://doi.org/10.1073/pnas.1421416112>
- Brooker, R. W. (2006). Plant – plant interactions and environmental change. *New Phytologist*, 171(2), 271–284. <https://doi.org/10.1111/j.1469-8137.2006.01752.x>
- Brooker, R.W. & Callaghan, T.V. (1998). The balance between positive and negative plant interactions and its relationship to environmental gradients: A model. *Oikos*, 81, 196–207.
- Bruno, J. F., Stachowicz, J. J., & Bertness, M. D. (2003). Inclusion of facilitation into ecological theory. *Trends in Ecology and Evolution*, 18(3), 119–125. [https://doi.org/10.1016/S0169-5347\(02\)00045-9](https://doi.org/10.1016/S0169-5347(02)00045-9)

- Bukowski, A. R., & Petermann, J. S. (2014). Intraspecific plant-soil feedback and intraspecific overyielding in *Arabidopsis thaliana*. *Ecology and Evolution*, 4(12), 2533–2545. <https://doi.org/10.1002/ece3.1077>
- Bukowski, A. R., Schittko, C., & Petermann, J. S. (2018). The strength of negative plant–soil feedback increases from the intraspecific to the interspecific and the functional group level. *Ecology and Evolution*, 8(4), 2280–2289. <https://doi.org/10.1002/ece3.3755>
- Burghardt, L. T., Epstein, B., & Tiffin, P. (2019). Legacy of prior host and soil selection on rhizobial fitness in planta. *Evolution*, 73(9), 2013–2023. <https://doi.org/10.1111/evo.13807>
- Burt, A., & Bell, G. (1992). Tests of sib diversification theories of outcrossing in *Impatiens capensis*: Effects of inbreeding and neighbour relatedness on production and infestation. *Journal of Evolutionary Biology*, 5(4), 575–588. <https://doi.org/10.1046/j.1420-9101.1992.5040575.x>
- Cahill, J.F., Kembel, S. W., & Gustafson, D. J. (2005). Differential genetic influences on competitive effect and response in *Arabidopsis thaliana*. *Journal of Ecology*, 93(5), 958–967. <https://doi.org/10.1111/j.1365-2745.2005.01013.x>
- Cahill, J. F., McNickle, G. G., Haag, J. J., Lamb, E. G., Nyanumba, S. M., & Clair, C. C. S. (2010). Plants integrate information about nutrients and neighbors. *Science*, 328(5986), 1657. <https://doi.org/10.1126/science.1189736>
- Callaway, R. M. (1995). Positive interactions among plants. *Botanical Review*, 61, 306–349.
- Callaway, R. M., and L. King. (1996). Oxygenation of the soil rhizosphere by *Typha latifolia* and its facilitative effects on other species. *Ecology*, 77, 1189–1195.
- Callaway, R. M., & Walker, L. R. (1997). Competition and facilitation: A synthetic approach to interactions in plant communities. *Ecology*, 78(7), 1958–1965. [https://doi.org/10.1890/0012-9658\(1997\)078\[1958:CAFASA\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1997)078[1958:CAFASA]2.0.CO;2)
- Chaney, L., & Baucom, R. S. (2014). The costs and benefits of tolerance to competition in *Ipomoea purpurea*, the common morning glory. *Evolution*, 68(6), 1698–1709. <https://doi.org/10.1111/evo.12383>
- Chapin, E S., III., L. R. Walker, C. L. Fastie, and L. C. Sharman. (1994). Mechanisms of primary succession following deglaciation at Glacier Bay, Alaska. *Ecological Monographs*. 64, 149–175.

- Chawade, A., Van Ham, J., Blomquist, H., Bagge, O., Alexandersson, E., & Ortiz, R. (2019). High-throughput field-phenotyping tools for plant breeding and precision agriculture. *Agronomy*, 9(5), 1–18. <https://doi.org/10.3390/agronomy9050258>
- Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics*, 31, 343–366. <https://doi.org/10.1146/annurev.ecolsys.31.1.343>
- Collins, A. R., Beckage, B., & Molofsky, J. (2018). Small-scale genotypic richness stabilizes plot biomass and increases phenotypic variance in the invasive grass *Phalaris arundinacea*. *Journal of Plant Ecology*, 11(1), 47–55. <https://doi.org/10.1093/jpe/rtx056>
- Corliss, C. T., & Sultan, S. E. (2016). Evolutionary potential for increased invasiveness: High-performance *Polygonum cespitosum* genotypes are competitively superior in full sun. *American Journal of Botany*, 103(2), 348–354. <https://doi.org/10.3732/ajb.1500306>
- Creissen, H. E., Jorgensen, T. H., & Brown, J. K. M. (2013). Stabilization of yield in plant genotype mixtures through compensation rather than complementation. *Annals of Botany*, 112(7), 1439–1447. <https://doi.org/10.1093/aob/mct209>
- Crepy, M. A., & Casal, J. J. (2015). Photoreceptor-mediated kin recognition in plants. *New Phytologist*, 205(1), 329–338. <https://doi.org/10.1111/nph.13040>
- Crutsinger, G.M., Collins, M.D., Fordyce, J.A., Gompert, Z., Nice, C.C. & Sanders, N.J. (2006). Plant genotypic diversity predicts community structure and governs an ecosystem process. *Science*, 313, 966–968.
- Crutsinger, G. M., Souza, L., & Sanders, N. J. (2008). Intraspecific diversity and dominant genotypes resist plant invasions. *Ecology Letters*, 11(1), 16–23. <https://doi.org/10.1111/j.1461-0248.2007.01118.x>
- Donohue, K. (2003). The influence of neighbor relatedness on multilevel selection in the Great Lakes sea rocket. *American Naturalist*, 162(1), 77–92. <https://doi.org/10.1086/375299>
- Dormann, C. F., & Brooker, R. W. (2002). Facilitation and competition in the high Arctic: The importance of the experimental approach. *Acta Oecologica*, 23(5), 297–301. [https://doi.org/10.1016/S1146-609X\(02\)01158-X](https://doi.org/10.1016/S1146-609X(02)01158-X)
- Drummond, E. B. M., & Vellend, M. (2012). Genotypic diversity effects on the performance of *Taraxacum officinale* populations increase with time and environmental favorability. *PLoS ONE*, 7(2). <https://doi.org/10.1371/journal.pone.0030314>

- Dudley, S. A., & File, A. L. (2007). Kin recognition in an annual plant. *Biology Letters*, 3(4), 435–438. <https://doi.org/10.1098/rsbl.2007.0232>
- Ehlers, B. K., Damgaard, C. F., & Laroche, F. (2016). Intraspecific genetic variation and species coexistence in plant communities. *Biology Letters*, 12(1). <https://doi.org/10.1098/rsbl.2015.0853>
- Ehlers, B. K., David, P., Damgaard, C. F., & Lenormand, T. (2016). Competitor relatedness, indirect soil effects and plant coexistence. *Journal of Ecology*, 104(4), 1126–1135. <https://doi.org/10.1111/1365-2745.12568>
- Espeland, E. K., & Rice, K. J. (2007). Facilitation across stress gradients: The importance of local adaptation. *Ecology*, 88(9), 2404–2409. <https://doi.org/10.1890/06-1217.1>
- Falik, O., de Kroon, H., & Novoplansky, A. (2006). Physiologically-mediated self/nonsel root discrimination in *Trifolium repens* has mixed effects on plant performance. *Plant Signaling and Behavior*, 1(3), 116–121. <https://doi.org/10.4161/psb.1.3.2639>
- File, A. L., Klironomos, J., Maherali, H., & Dudley, S. A. (2012). Plant kin recognition enhances abundance of symbiotic microbial partner. *PLoS ONE*, 7(9), 15–17. <https://doi.org/10.1371/journal.pone.0045648>
- Fitzpatrick, C. R., Mustafa, Z., & Viliunas, J. (2019). Soil microbes alter plant fitness under competition and drought. *Journal of Evolutionary Biology*, 32(5), 438–450. <https://doi.org/10.1111/jeb.13426>
- Fournier-Level, A., Korte, A., Cooper, M. D., Nordborg, M., Schmitt, J., and Wilczek, A. M. (2011). A map of local adaptation in *Arabidopsis thaliana*. *Science*, 334, 86–89. doi: 10.1126/science.1209271
- Frachon, L., Bartoli, C., Carrère, S., Bouchez, O., Chaubet, A., Gautier, M., Roby, D., Roux, F. (2018). A genomic map of climate adaptation in *Arabidopsis thaliana* at a micro-geographic scale. *Frontiers in Plant Science*, 9, 1–15. <https://doi.org/10.3389/fpls.2018.00967>
- Frachon, L., Libourel, C., Villoutreix, R., Carrère, S., Glorieux, C., Huard-Chauveau, C., Navascues, M., Gay, L., Vitalis, R., Baron, E., Amsellem, L., Bouchez, O., Vidal, M., Le Corre, V., Roby, D., Bergelson, J., Roux, F. (2017). Intermediate degrees of synergistic pleiotropy drive adaptive evolution in ecological time. *Nature Ecology & Evolution*, 1, 1551–1561. <https://doi.org/10.1038/s41559-017-0297-1>

- Frachon, L., Mayjonade, B., Bartoli, C., Hautekèete, N. C., & Roux, F. (2019). Adaptation to plant communities across the genome of *Arabidopsis thaliana*. *Molecular Biology and Evolution*, 36(7), 1442–1456. <https://doi.org/10.1093/molbev/msz078>
- Fulgione, A., Koornneef, M., Roux, F., Hermisson, J., & Hancock, A. M. (2018). Madeiran *Arabidopsis thaliana* reveals ancient long-range colonization and clarifies demography in Eurasia. *Molecular Biology and Evolution*, 35(3), 564–574. <https://doi.org/10.1093/molbev/msx300>
- Gianoli, E. (2015). The behavioural ecology of climbing plants. *AoB PLANTS*, 7(1), 1–11. <https://doi.org/10.1093/aobpla/plv013>
- Gilman, S. E., Urban, M. C., Tewksbury, J., Gilchrist, G. W., & Holt, R. D. (2010). A framework for community interactions under climate change. *Trends in Ecology and Evolution*, 25(6), 325–331. <https://doi.org/10.1016/j.tree.2010.03.002>
- Goldberg, D. E., & Barton, A. M. (1992). Patterns and consequences of interspecific competition in natural communities - a review of field experiments with plants. *American Naturalist*, 139(4), 771–801. [https://doi.org/Doi 10.1086/285357](https://doi.org/Doi%2010.1086/285357)
- Goodnight, C. J. (1985). The influence of environmental variation on group and individual selection in a cress. *Evolution*, 39(3), 545. <https://doi.org/10.2307/2408652>
- Griffing, B. (1989). Genetic analysis of plant mixtures. *Genetics*, 122(4), 943–94356.
- Hancock, A. M., Brachi, B., Faure, N., Horton, M. W., Jarymowycz, L. B., Sperone, F. G., Toomijan, C., Roux, F., Bergelson, J (2011). Adaptation to climate across the *Arabidopsis thaliana* genome. *Science* 334, 83–86. doi: 10.1126/science.1209244
- Hamilton, W. D. (2017). The genetical evolution of social behavior. I. *Group Selection*, 23–43. <https://doi.org/10.4324/9780203790427-4>
- Harper, J.L. (1977). Population biology of plants. *Academic Press, London*.
- Hawkins, A. P., & Crawford, K. M. (2018). Interactions between plants and soil microbes may alter the relative importance of intraspecific and interspecific plant competition in a changing climate. *AoB PLANTS*, 10(4), 1–11. <https://doi.org/10.1093/aobpla/ply039>
- Holmgren, M., M. Scheffer, and M. A. Huston. (1997). The interplay of facilitation and competition in plant communities. *Ecology*, 78(7), 1966–1975.
- Horton, M. W., Hancock, A. M., Huang, Y. S., Toomajian, C., Atwell, S., Auton, A., Mulyati, N.W., Platt, A., Sperone, Vilhjalmsson, B.J. Nordborg, M., Borevitz, J.O., Bergelson, J

- (2012). Genome-wide patterns of genetic variation in worldwide *Arabidopsis thaliana* accessions from the RegMap panel. *Nature Genetics*, 44(2), 212–216. <https://doi.org/10.1038/ng.1042>. [Genome-wide](https://doi.org/10.1038/ng.1042)
- Huber, H., During, H. J., de Bruin, F. B., Vermeuinen, P. J., & Anten, N. P. R. (2016). Genotypic and phenotypic diversity does not affect productivity and drought response in competitive stands of *Trifolium repens*. *Frontiers in Plant Science*, 7, 1–15. <https://doi.org/10.3389/fpls.2016.00364>
- Hughes, A. R., Inouye, B. D., Johnson, M. T. J., Underwood, N., & Vellend, M. (2008). Ecological consequences of genetic diversity. *Ecology Letters*, 11(6), 609–623. <https://doi.org/10.1111/j.1461-0248.2008.01179.x>
- Jie, L., Xingliang, X., & Tao, L. (2017). Kin interactions of *Arabidopsis* based on the integrated performance of plants. *Journal of Resources and Ecology*, 8(2), 185–190. <https://doi.org/10.5814/j.issn.1674-764x.2017.02.009>
- Johnson, M.G., Tingey, D.T., Phillips, D.L. and Storm, M.J. (2001) Advancing fine root research with minirhizotrons. *Environmental and Experimental Botany* 45, 263–289.
- Jorgensen, T. H., & Emerson, B. C. (2008). Functional variation in a disease resistance gene in populations of *Arabidopsis thaliana*. *Molecular Ecology*, 17(22), 4912–4923. <https://doi.org/10.1111/j.1365-294X.2008.03960.x>
- Joseph, B., Lau, L., & Kliebenstein, D. J. (2015). Quantitative variation in responses to root spatial constraint within *Arabidopsis thaliana*. *Plant Cell*, 27(8), 2227–2243. <https://doi.org/10.1105/tpc.15.00335>
- Karban, R., & Shiojiri, K. (2009). Self-recognition affects plant communication and defense. *Ecology Letters*, 12(6), 502–506. <https://doi.org/10.1111/j.1461-0248.2009.01313.x>
- Karban, R., Shiojiri, K., Ishizaki, S., Wetzal, W. C., & Evans, R. Y. (2013). Kin recognition affects plant communication and defence. *Proceedings of the Royal Society B: Biological Sciences*, 280(1756), 1–5. <https://doi.org/10.1098/rspb.2012.3062>
- Karban, R., Wetzal, W. C., Shiojiri, K., Ishizaki, S., Ramirez, S. R., & Blande, J. D. (2014). Deciphering the language of plant communication: Volatile chemotypes of sagebrush. *Journal of Physiology*, 204(2), 380–385. <https://doi.org/10.1111/nph.12887>
- Kardol, P., Bezemer, T.M. & Van der Putten, W.H. (2006). Temporal variation in plant–soil feedbacks controls succession. *Ecology Letters*, 9, 1080–1088.

- Kardol, P., Cornips, N.J., Van Kempen, M.M.L., Bakx-Schotman, J.M.T. & Van der Putten, W.H. (2007). Microbe-mediated plant–soil feedbacks causes historical contingency effects in plant community assembly. *Ecological Monographs*, 77, 147–162.
- Keddy, P.A. (2015) Competition in plant communities. *Oxford Bibliographies in Ecology*. <https://doi.org/10.1093/obo/9780199830060-0009>
- Kellman, M., and M. Kading. (1992). Facilitation of tree seed-ling establishment in a sand dune succession. *Journal of Vegetation Science*, 3, 679–688.
- Keurentjes, J. J. B., Bentsink, L., Alonso-Blanco, C., Hanhart, C. J., Vries, H. B. De, Effgen, S., Vreugdenhil, D., Koornneef, M. (2007). Development of a near-isogenic line population of *Arabidopsis thaliana* and comparison of mapping power with a recombinant inbred line population. *Genetics*, 175(2), 891–905. <https://doi.org/10.1534/genetics.106.066423>
- Klein, S. L. (2003). Parasite manipulation of the proximate mechanisms that mediate social behavior in vertebrates. *Physiology and Behavior*, 79(3), 441–449. [https://doi.org/10.1016/S0031-9384\(03\)00163-X](https://doi.org/10.1016/S0031-9384(03)00163-X)
- Klironomos, J.N. (2002). Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature*, 417, 67–70.
- Kotowska, A. M., Cahill, J. F., & Keddie, B. A. (2010). Plant genetic diversity yields increased plant productivity and herbivore performance. *Journal of Ecology*, 98(1), 237–245. <https://doi.org/10.1111/j.1365-2745.2009.01606.x>
- Kramer, J., & Meunier, J. (2016). Kin and multilevel selection in social evolution: A never-ending controversy? *F1000Research*, 5(0). <https://doi.org/10.12688/F1000RESEARCH.8018.1>
- Kronholm, I., Picó, F. X., Alonso-Blanco, C., Goudet, J., and de Meaux, J. (2012). Genetic basis of adaptation in *Arabidopsis thaliana*: local adaptation at the seed dormancy QTL DOG1. *Evolution*. 66, 2287–2302. doi: 10.1111/j.1558-5646.2012.01590.x
- Latzel, V., Allan, E., Bortolini Silveira, A., Colot, V., Fischer, M., & Bossdorf, O. (2013). Epigenetic diversity increases the productivity and stability of plant populations. *Nature Communications*, 4, 1–7. <https://doi.org/10.1038/ncomms3875>
- Le Corre, V. (2005). Variation at two flowering time genes within and among populations of *Arabidopsis thaliana*: comparison with markers and traits. *Molecular Ecology*, 14, 4181–4192. doi: 10.1111/j.1365-294X.2005.02722.x

- Lewin-Epstein, O., Aharonov, R., & Hadany, L. (2017). Microbes can help explain the evolution of host altruism. *Nature Communications*, 8, 1–7. <https://doi.org/10.1038/ncomms14040>
- Li, J., & Xu, X. (2018). Relatives growing together: pair density and kinship. *Plant Ecology*, 219(2), 159–168. <https://doi.org/10.1007/s11258-017-0785-z>
- Linhart, Y. B. (1988). Intrapopulation differentiation in annual plants. III. The contrasting effects of intra- and interspecific competition. *Evolution*, 42(5), 1047. <https://doi.org/10.2307/2408920>
- Loreau, M. (2004). Does functional redundancy exist? *Oikos*, 104, 606–611
- Maestre, F. T., Valladares, F., & Reynolds, J. F. (2005). Is the change of plant-plant interactions with abiotic stress predictable? A meta-analysis of field results in arid environments. *Journal of Ecology*, 93(4), 748–757. <https://doi.org/10.1111/j.1365-2745.2005.01017.x>
- Mahall, B. E., & Callaway, R. M. (1991). Root communication among desert shrubs. *Proceedings of the National Academy of Sciences of the United States of America*, 88(3), 874–876. <https://doi.org/10.1073/pnas.88.3.874>
- Mahall, B. E., & Callaway, R. M. (1996). Effects of regional origin and genotype on intraspecific root communication in the desert shrub *Ambrosia dumosa* (Asteraceae). *American Journal of Botany*, 83(1), 93–98. <https://doi.org/10.2307/2445959>
- Markham, J., & Halwas, S. (2011). Effect of neighbour presence and soil volume on the growth of *Andropogon gerardii* Vitman. *Plant Ecology and Diversity*, 4(2–3), 265–268. <https://doi.org/10.1080/17550874.2011.618515>
- Martorell, C., & Freckleton, R. P. (2014). Testing the roles of competition, facilitation and stochasticity on community structure in a species-rich assemblage. *Journal of Ecology*, 102(1), 74–85. <https://doi.org/10.1111/1365-2745.12173>
- Masclaux, F. G., Bruessow, F., Schweizer, F., Gouhier-Darimont, C., Keller, L., & Reymond, P. (2012). Transcriptome analysis of intraspecific competition in *Arabidopsis thaliana* reveals organ-specific signatures related to nutrient acquisition and general stress response pathways. *BMC Plant Biology*, 12(1), 227. <https://doi.org/10.1186/1471-2229-12-227>
- Masclaux, F., Hammond, R. L., Meunier, J., Gouhier-Darimont, C., Keller, L., & Reymond, P. (2010). Competitive ability not kinship affects growth of *Arabidopsis thaliana* accessions. *New Phytologist*, 185(1), 322–331. <https://doi.org/10.1111/j.1469-8137.2009.03057.x>

- Mazzoleni, S., Bonanomi, G., Incerti, G., Chiusano, M. L., Termolino, P., Mingo, A., Senatore, M., Giannino, F., Carten, F., Rietkerk, M., Lanzotti, V. (2015). Inhibitory and toxic effects of extracellular self-DNA in litter: A mechanism for negative plant-soil feedbacks? *New Phytologist*, 205(3), 1195–1210. <https://doi.org/10.1111/nph.13121>
- Milla, R., Del Burgo, A. V., Escudero, A., & Iriondo, J. M. (2012). Kinship rivalry does not trigger specific allocation strategies in *Lupinus angustifolius*. *Annals of Botany*, 110(1), 165–175. <https://doi.org/10.1093/aob/mcs093>
- Murphy, G. P., & Dudley, S. A. (2009). Kin recognition: Competition and cooperation in *Impatiens* (Balsaminaceae). *American Journal of Botany*, 96(11), 1990–1996. <https://doi.org/10.3732/ajb.0900006>
- Mutic, J. J., & Wolf, J. B. (2007). Indirect genetic effects from ecological interactions in *Arabidopsis thaliana*. *Molecular Ecology*, 16(11), 2371–2381. <https://doi.org/10.1111/j.1365-294X.2007.03259.x>
- Ninkovic, V. (2003). Volatile communication between barley plants affects biomass allocation. *Journal of Experimental Botany*, 54(389), 1931–1939. <https://doi.org/10.1093/jxb/erg192>
- Ninkovic, V., Rensing, M., Dahlin, I., & Markovic, D. (2019). Who is my neighbor? Volatile cues in plant interactions. *Plant Signaling and Behavior*, 14(9). <https://doi.org/10.1080/15592324.2019.1634993>
- Nowak, M. A. (2006). Fiver rules for the evolution of cooperation. *Science*, 314(5805), 1560–1563. <https://doi.org/10.1126/science.1133755>.Five
- Nowak, M. A., Tarnita, C. E., & Wilson, E. O. (2010). The evolution of eusociality. *Nature*, 466(7310), 1057–1062. <https://doi.org/10.1038/nature09205>
- Ormeño, E., Bousquet-Mélou, A., Mévy, J. P., Greff, S., Robles, C., Bonin, G., & Fernandez, C. (2007). Effect of intraspecific competition and substrate type on terpene emissions from some Mediterranean plant species. *Journal of Chemical Ecology*, 33(2), 277–286. <https://doi.org/10.1007/s10886-006-9219-4>
- Padilla, F. M., & Pugnaire, F. I. (2006). The role of nurse plants in restoration. *Frontiers in Ecology and the Environment*, 4, 196–202.
- Palmer, A. G., Ali, M., Yang, S., Parchami, N., Bento, T., Mazzella, A., Oni, M., Riley, M.C., Schneider, K., Massa, N. (2016). Kin recognition is a nutrient-dependent inducible

- phenomenon. *Plant Signaling and Behavior*, 11(9).  
<https://doi.org/10.1080/15592324.2016.1224045>
- Petermann, J. S., Fergus, A. J. F., Turnbull, L. A., and Schmid, B. (2008). Janzen Connell effects are widespread and strong enough to maintain diversity. *Ecology* 89, 2399–2406. doi: 10.1890/07-2056.1
- Piotto, D. (2008). A meta-analysis comparing tree growth in monocultures and mixed plantations. *Forest Ecology and Management*, 255(3–4), 781–786.  
<https://doi.org/10.1016/j.foreco.2007.09.065>
- Platt, A., Horton, M., Huang, Y. S., Li, Y., Anastasio, A. E., Mulyati, N. W., [Agren, J.](#), [Bossdorf, O.](#), [Byers, D.](#), [Donohue, K.](#), [Dunning, M.](#), [Holub, E.B.](#), Hudson, A., [Le Corre, V.](#), [Loudet, O.](#), [Roux, F.](#), [Warthmann, N.](#), [Weigel, D.](#), [Rivero, L.](#), [Scholl, R.](#), [Nordborg, M.](#), [Bergelson, J.](#), Borevitz, J. O. (2010). The scale of population structure in *Arabidopsis thaliana*. *PLoS Genetics*, 6(2). <https://doi.org/10.1371/journal.pgen.1000843>
- Pugnaire, F I., P Haase, J. Puigdefabregas, M. Cueto, S. C. Clark, and L. D. Incoll. (1996). Facilitation and succession under the canopy of a leguminous shrub in a semiarid environment in southeast Spain. *Oikos*, 76, 455-464.
- Pugnaire, F. I., & Luque, M. T. (2001). Changes in plant interactions along a gradient of environmental stress. *Oikos*, 93(1), 42–49. <http://doi.org/10.1034/j.1600-0706.2001.930104.x>
- Queller, D. C. (2011). Expanded social fitness and Hamilton’s rule for kin, kith, and kind. *Proceedings of the National Academy of Sciences of the United States of America*, 108(SUPPL. 2), 10792–10799. <https://doi.org/10.1073/pnas.1100298108>
- Rautiainen, P., Koivula, K., & Hyvärinen, M. (2004). The effect of within-genet and between-genet competition on sexual reproduction and vegetative spread in *Potentilla anserina* ssp. *egedii*. *Journal of Ecology*, 92(3), 505–511. <https://doi.org/10.1111/j.0022-0477.2004.00878.x>
- Reinhart, K. O., & Callaway, R. M. (2006). Soil biota and invasive plants. *New Phytologist*, 170(3), 445–457. <https://doi.org/10.1111/j.1469-8137.2006.01715.x>
- Ronsheim, M. L., & Anderson, S. E. (2001). Population-level specificity in the plant-mycorrhizae association alters intraspecific interactions among neighboring plants. *Oecologia*, 128(1), 77–84. <https://doi.org/10.1007/s004420000625>

- Rousset F, Lion S. (2011). Much ado about nothing: Nowak et al.'s charge against inclusive fitness theory. *Journal of Evolutionary Biology*, 24(6),1386–1392
- Roux, F., & Bergelson, J. (2016). The genetics underlying natural variation in the biotic interactions of *Arabidopsis thaliana*: The challenges of linking evolutionary genetics and community ecology. *Current Topics in Developmental Biology*, 119, 111-156. <https://doi.org/10.1016/bs.ctdb.2016.03.001>
- Tomiolo, S., Thomas, C., Jespersen, M.K., Damgaard, C.F., Ehlers, B.K. (2019). Kin recognition and genetic variation for competitive ability in the annual legume *Medicago minima*. *bioRxiv*, 1–28. <https://doi.org/10.1101/695908>
- Schmid, B., Hector, A., Saha, P., & Loreau, M. (2008). Biodiversity effects and transgressive overyielding. *Journal of Plant Ecology*, 1(2), 95–102. <https://doi.org/10.1093/jpe/rtn011>
- Semchenko, M., John, E. A., & Hutchings, M. J. (2007). Effects of physical connection and genetic identity of neighbouring ramets on root-placement patterns in two clonal species. *New Phytologist*, 176(3), 644–654. <https://doi.org/10.1111/j.1469-8137.2007.02211.x>
- Semchenko, M., Saar, S., & Lepik, A. (2017). Intraspecific genetic diversity modulates plant-soil feedback and nutrient cycling. *New Phytologist*. <https://doi.org/10.1111/nph.14653>
- Shavit, A. (2004). Shifting values partly explain the debate over group selection. *Studies in History and Philosophy of Science Part C: Studies in History and Philosophy of Biological and Biomedical Sciences*, 35(4), 697–720. <https://doi.org/10.1016/j.shpsc.2004.09.007>
- Shi, J., Joshi, J., Tielbörger, K., Verhoeven, K. J. F., & Macel, M. (2018). Costs and benefits of admixture between foreign genotypes and local populations in the field. *Ecology and Evolution*, 8(7), 3675–3684. <https://doi.org/10.1002/ece3.3946>
- Singer, A., Travis, J. M. J., & Johst, K. (2013). Interspecific interactions affect species and community responses to climate shifts. *Oikos*, 122(3), 358–366. <https://doi.org/10.1111/j.1600-0706.2012.20465.x>
- Šmarda, P., Horová, L., Bureš, P., Hralová, I., & Marková, M. (2010). Stabilizing selection on genome size in a population of *Festuca pallens* under conditions of intensive intraspecific competition. *New Phytologist*, 187(4), 1195–1204. <https://doi.org/10.1111/j.1469-8137.2010.03335.x>

- Smit, C., Rietkerk, M., & Wassen, M. J. (2009). Inclusion of biotic stress (consumer pressure) alters predictions from the stress gradient hypothesis. *Journal of Ecology*, 97(6), 1215–1219. <https://doi.org/10.1111/j.1365-2745.2009.01555.x>
- Snell, R., & Aarssen, L. W. (2005). Life history traits in selfing versus outcrossing annuals: Exploring the “time-limitation” hypothesis for the fitness benefit of self-pollination. *BMC Ecology*, 5, 1–14. <https://doi.org/10.1186/1472-6785-5-2>
- Subrahmaniam, H. J., Libourel, C., Journet, E. P., Morel, J. B., Muños, S., Niebel, A., Raffael, S., Roux, F. (2018). The genetics underlying natural variation of plant–plant interactions, a beloved but forgotten member of the family of biotic interactions. *Plant Journal*, 93(4), 747–770. <https://doi.org/10.1111/tpj.13799>
- Subramanian, S., Han, L., Dutilleul, P., & Smith, D. L. (2015). Computed tomography scanning can monitor the effects of soil medium on root system development: An example of salt stress in corn. *Frontiers in Plant Science*, 6, 1–13. <https://doi.org/10.3389/fpls.2015.00256>
- Teste, F. P., Veneklaas, E. J., Dixon, K. W., & Lambers, H. (2014). Complementary plant nutrient-acquisition strategies promote growth of neighbour species. *Functional Ecology*, 28(4), 819–828. <https://doi.org/10.1111/1365-2435.12270>
- Thomas, S.C., and Bazzaz, F.A. (1993). The genetic component in plant size hierarchies : norms of reaction to density in a Polygonum species. *Ecological Society of America*, 63(3), 231–249.
- Tilman D. (1985). The resource-ratio hypothesis of plant succession. *The American Naturalist*, 125(6): 827-852.
- Tomimatsu, H., Nakano, K., Yamamoto, N., & Suyama, Y. (2014). Effects of genotypic diversity of *Phragmites australis* on primary productivity and water quality in an experimental wetland. *Oecologia*, 175(1), 163–172. <https://doi.org/10.1007/s00442-014-2896-8>
- Torices, R., Gómez, J. M., & Pannell, J. R. (2018). Kin discrimination allows plants to modify investment towards pollinator attraction. *Nature Communications*, 9(1), 1–6. <https://doi.org/10.1038/s41467-018-04378-3>
- Turkington, R. & Harper, J.L. (1979) The growth, distribution and neighbour relationships of *Trifolium repens* in a permanent pasture. IV. Fine-scale biotic differentiation. *Journal of Ecology*, 67, 245 –254

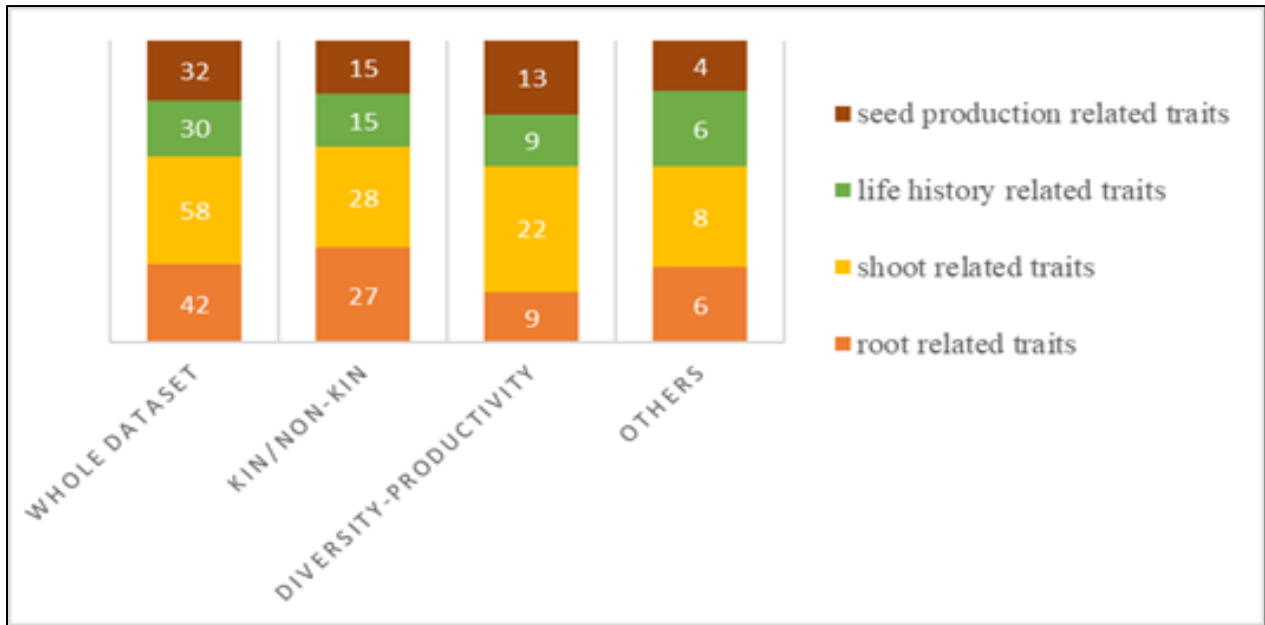
- Turkington, R. (1989). The growth, distribution and neighbour relationships of *Trifolium repens* in a permanent pasture. V. The coevolution of competitors. *The Journal of Ecology*, 77(3), 717. <https://doi.org/10.2307/2260981>
- Vandermeer J. (1981). The interference production principle: ecological theory for agriculture. *BioScience*, 31, 361–64.
- Wagg, C., Boller, B., Schneider, S., Widmer, F., & van der Heijden, M. G. A. (2015). Intraspecific and intergenerational differences in plant-soil feedbacks. *Oikos*, 124(8), 994–1004. <https://doi.org/10.1111/oik.01743>
- Walker, L. R., and P M. Vitousek. (1991). An invader alters germination and growth of a native dominant tree in Hawaiï. *Ecology*, 72, 1449-1455.
- Wendling, M., Büchi, L., Amossé, C., Jeangros, B., Walter, A., & Charles, R. (2017). Specific interactions leading to transgressive overyielding in cover crop mixtures. *Agriculture, Ecosystems and Environment*, 241, 88–99. <https://doi.org/10.1016/j.agee.2017.03.003>
- Willis, C. G., Brock, M. T., & Weinig, C. (2010). Genetic variation in tolerance of competition and neighbour suppression in *Arabidopsis thaliana*. *Journal of Evolutionary Biology*, 23(7), 1412–1424. <https://doi.org/10.1111/j.1420-9101.2010.02003.x>
- Willson, M. F., Hoppes, W. G., Goldman, D. A., Thomas, P. A., & Bothwell, J. L. (2016). Sibling competition in plants : an experimental study. *The American Society of Naturalists*, 129(2), 304–311.
- Wolf, J. B., Mutic, J. J., & Kover, P. X. (2011). Functional genetics of intraspecific ecological interactions in *Arabidopsis thaliana*. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 366(1569), 1358–1367. <https://doi.org/10.1098/rstb.2010.0239>
- Wuest, S. E., & Niklaus, P. A. (2018). A plant biodiversity effect resolved to a single chromosomal region. *Nature Ecology and Evolution*, 2(12), 1933–1939. <https://doi.org/10.1038/s41559-018-0708-y>
- Yang, L., Callaway, R. M., & Atwater, D. Z. (2015). Root contact responses and the positive relationship between intraspecific diversity and ecosystem productivity. *AoB PLANTS*, 7(1), 1–8. <https://doi.org/10.1093/aobpla/plv053>
- Yuan, J., Zhao, J., Wen, T., Zhao, M., Li, R., Goossens, P., Huang, Q., Bai, Y., Vivanco, J.M., Kowalchuk, G.A., Berendsen, R.L., Shen, Q. (2018). Root exudates drive the soil-borne

legacy of aboveground pathogen infection. *Microbiome*, 6(1), 1–12.  
<https://doi.org/10.1186/s40168-018-0537-x>

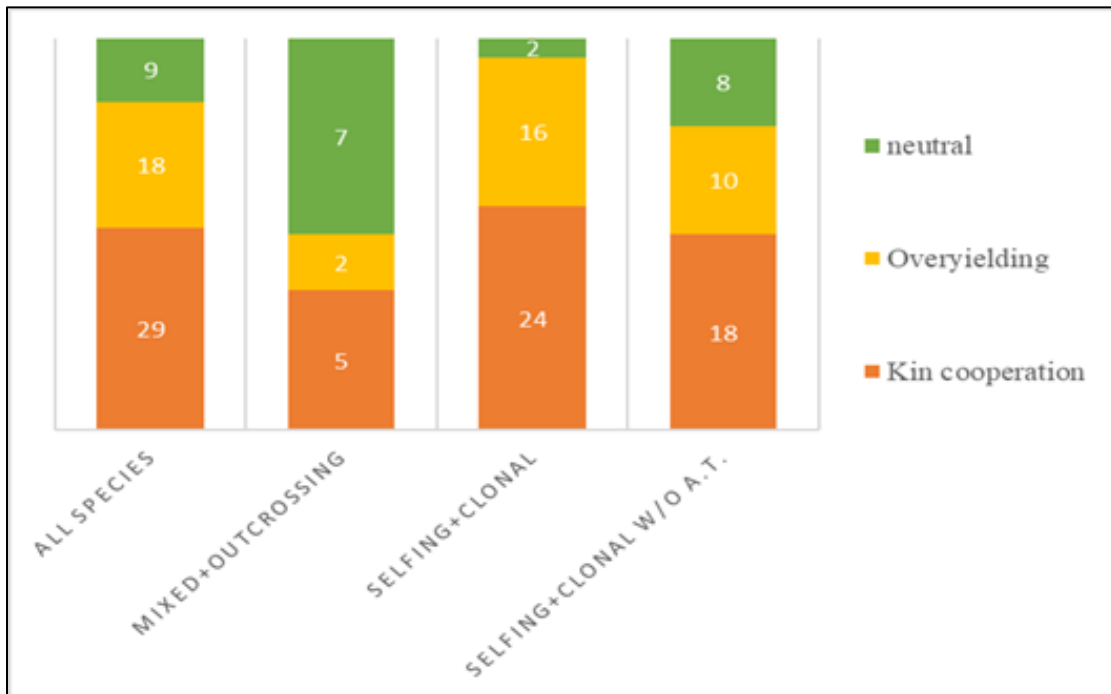
Zhang, R., & Tielbörger, K. (2019). Facilitation from an intraspecific perspective – stress tolerance determines facilitative effect and response in plants. *New Phytologist*, 221(4), 2203–2212.  
<https://doi.org/10.1111/nph.15528>

Zhang, Y., Chen, H. Y. H., & Reich, P. B. (2012). Forest productivity increases with evenness, species richness and trait variation: A global meta-analysis. *Journal of Ecology*, 100(3), 742–749. <https://doi.org/10.1111/j.1365-2745.2011.01944>.

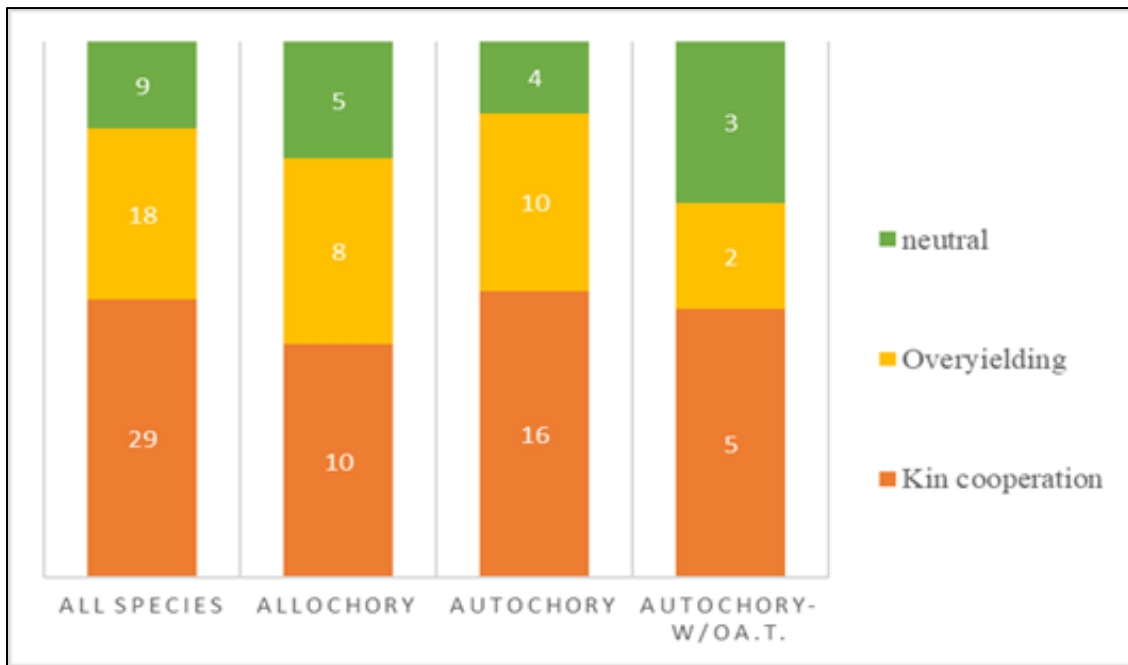
**Supplementary Figure 1:** Stacked barplots illustrating the distribution of studies based on relative proportion of trait categories measured in different rationales used in studying GXG interactions.



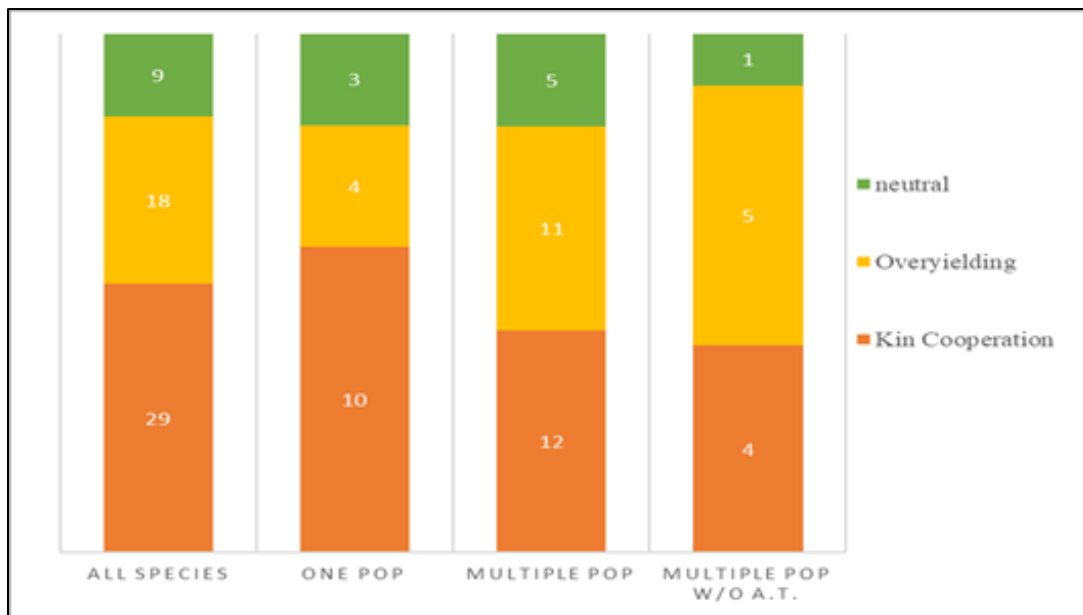
**Supplementary Figure 2:** Stacked barplots illustrating the different outcomes of GxG based on the breeding system of species studied (selfing and clonal species combined while outcrossing combined with mixed breeding: with and without including *Arabidopsis thaliana*)



**Supplementary Figure 3:** Stacked barplots illustrating the different outcomes of GxG based on mode of seed dispersal of the studied species (with and without including *Arabidopsis thaliana*).



**Supplementary Figure 4:** Stacked barplots illustrating the different outcomes of GxG based on the geographic scale of sampling (genotypes collected from one population vs multiple populations: with and without including *Arabidopsis thaliana*).



**Supplementary Table 1: Survey of GxG interaction studies in herbaceous wild plant species.** The first six columns indicate the list of different plant species studied, their botanical family, main breeding system, life cycle and their natural seed dispersal mode. Next six columns describe the genetic material used including the type of main type of genetic variation studied, number of genetic lines sampled, number of populations sampled, the scale of sampling, whether the genetic differences between the lines were tested or assumed, and the habitats from which these lines were sampled from. Following 15 columns are divided to describe the experimental design of the studies including the main rationale, whether the GxG interactions were tested directly or indirectly, the experimental design and conditions under which the experiments were performed. Additionally, details about the specific treatments applied and the traits measured are listed. Next three columns details the main ecological questions answered in each study, major findings with respect to intraspecific plant-plant interactions and the main type of positive interaction observed in each experimental study. The last three columns list the reference name, year of publication and the journal of publication.

Supplementary Table 1

Number	Species studied	Bifurcal family	Main breeding system	Life span	Seed dispersal mode	Height of tree	Number of populations	Number of individuals sampled	Sexual dimorphism	Genetic diversity	Habitat	Mimicry	Direct effect of CO <sub>2</sub> on fitness	Experimental design		Likelihood ratio test	Spatio-temporal correlation	Percent of treatment	Thinned density	Measurements		Total number of trials	Ecological factors assessed in reciprocal transplant	Key year of treatment	Year	Journal
														Block	Plot					Block	Plot					
1	<i>Arabidopsis thaliana</i>	Brassicaceae	selfing	annual	autochthonous	neutral	2	100	NR	NR	NR	Others	direct	x	lab	no/na	yes	above	density	reduced	1	Individuals of each population were transplanted to a common garden. Environmental variation was assessed in a common garden across genetic backgrounds.	2007	Evolution		
2	<i>Phytolacca americana</i>	Phytolaccaceae	selfing	perennial	autochthonous	neutral	10	100	NR	NR	NR	Within vs. among	direct	x	lab	no/na	yes	above	density	reduced	1	Individuals of each population were transplanted to a common garden. Environmental variation was assessed in a common garden across genetic backgrounds.	2007	Evolution		
3	<i>Malva sylvestris</i>	Malvaceae	selfing	annual	autochthonous	neutral	10	100	NR	NR	NR	Within vs. among	direct	x	lab	no/na	yes	above	density	reduced	5	Phenotypic plasticity was assessed in a common garden across genetic backgrounds.	2007	Evolution		
4	<i>Solanum elaeagnifolium</i>	Solanaceae	mixed	perennial	autochthonous	neutral	10	100	NR	NR	NR	Within vs. among	direct	x	lab	no/na	yes	above	density	reduced	4	Phenotypic plasticity was assessed in a common garden across genetic backgrounds.	2007	Evolution		
5	<i>Ipomoea pes-caprae</i>	Solanaceae	mixed	perennial	autochthonous	neutral	10	100	NR	NR	NR	Within vs. among	direct	x	lab	no/na	yes	above	density	reduced	4	Phenotypic plasticity was assessed in a common garden across genetic backgrounds.	2007	Evolution		
6	<i>Hydrocotyle pinnatifida</i>	Utriculariaceae	selfing	annual	autochthonous	neutral	7	100	NR	NR	NR	Others	direct	x	lab	no/na	yes	above	density	reduced	8	Genetic diversity was assessed in a common garden across genetic backgrounds.	2007	Evolution		
7	<i>Arabidopsis thaliana</i>	Brassicaceae	selfing	annual	autochthonous	neutral	2	100	NR	NR	NR	diversity/variability/relationship	direct	x	lab	no/na	yes	above	temperature, humidity	reduced	1	Genetic diversity was assessed in a common garden across genetic backgrounds.	2007	Evolution		
8	<i>Phytolacca americana</i>	Phytolaccaceae	mixed	perennial	autochthonous	neutral	9	100	NR	NR	NR	Within vs. among	direct	x	lab	no/na	yes	above	density	reduced	4	Genetic diversity was assessed in a common garden across genetic backgrounds.	2007	Evolution		
9	<i>Achillea millefolium</i>	Asteraceae	mixed	perennial	autochthonous	neutral	10	100	NR	NR	NR	Within vs. among	direct	x	lab	no/na	yes	above	density	reduced	1	Genetic diversity was assessed in a common garden across genetic backgrounds.	2007	Evolution		
10	<i>Lythrum salicaria</i>	Lythraceae	mixed	perennial	autochthonous	neutral	10	100	NR	NR	NR	Within vs. among	direct	x	lab	no/na	yes	above	density	reduced	1	Genetic diversity was assessed in a common garden across genetic backgrounds.	2007	Evolution		
11	<i>Impatiens capensis</i>	Balanophoraceae	mixed	annual	autochthonous	neutral	28	100	NR	NR	NR	Within vs. among	direct	x	lab	no/na	yes	above	density	reduced	3	Genetic diversity was assessed in a common garden across genetic backgrounds.	2007	Evolution		
12	<i>Impatiens capensis</i>	Balanophoraceae	mixed	annual	autochthonous	neutral	15	100	NR	NR	NR	diversity/variability/relationship	direct	x	lab	no/na	yes	above	density	reduced	5	Genetic diversity was assessed in a common garden across genetic backgrounds.	2007	Evolution		
13	<i>Polypogon monspeliensis</i>	Polygonaceae	mixed	annual	autochthonous	neutral	25	100	NR	NR	NR	diversity/variability/relationship	direct	x	lab	no/na	yes	above	density	reduced	6	Genetic diversity was assessed in a common garden across genetic backgrounds.	2007	Evolution		
14	<i>Achillea millefolium</i>	Asteraceae	mixed	perennial	autochthonous	neutral	10	100	NR	NR	NR	Within vs. among	direct	x	lab	no/na	yes	above	density	reduced	1	Genetic diversity was assessed in a common garden across genetic backgrounds.	2007	Evolution		
15	<i>Arabidopsis thaliana</i>	Brassicaceae	selfing	annual	autochthonous	neutral	5	multiple	regional	NR	NR	diversity/variability/relationship	direct	x	lab	no/na	yes	above	density	reduced	3	Genetic diversity was assessed in a common garden across genetic backgrounds.	2007	Evolution		
16	<i>Asteraceae</i>	Asteraceae	selfing	perennial	autochthonous	neutral	5	100	NR	NR	NR	diversity/variability/relationship	direct	x	lab	no/na	yes	above	density	reduced	2	Genetic diversity was assessed in a common garden across genetic backgrounds.	2007	Evolution		
17	<i>Galium aparine</i>	Brassicaceae	selfing	annual	autochthonous	neutral	14	100	NR	NR	NR	Others	direct	x	lab	no/na	yes	above	density	reduced	4	Genetic diversity was assessed in a common garden across genetic backgrounds.	2007	Evolution		
18	<i>Phytolacca americana</i>	Rubaceae	mixed	perennial	autochthonous	neutral	50	14	regional	NR	NR	Within vs. among	direct	x	lab	no/na	yes	above	density	reduced	4	Genetic diversity was assessed in a common garden across genetic backgrounds.	2007	Evolution		
19	<i>Arabidopsis thaliana</i>	Brassicaceae	selfing	annual	autochthonous	neutral	12	100	NR	NR	NR	Within vs. among	direct	x	lab	no/na	yes	above	density	reduced	2	Genetic diversity was assessed in a common garden across genetic backgrounds.	2007	Evolution		
20	<i>Solanum elaeagnifolium</i>	Asteraceae	selfing	perennial	autochthonous	neutral	21	100	NR	NR	NR	diversity/variability/relationship	direct	x	lab	no/na	yes	above	density	reduced	1	Genetic diversity was assessed in a common garden across genetic backgrounds.	2007	Evolution		
21	<i>Lythrum salicaria</i>	Rubaceae	mixed	perennial	autochthonous	neutral	8	100	NR	NR	NR	Within vs. among	direct	x	lab	no/na	yes	above	density	reduced	3	Genetic diversity was assessed in a common garden across genetic backgrounds.	2007	Evolution		
22	<i>Arabidopsis thaliana</i>	Brassicaceae	selfing	annual	autochthonous	neutral	10	100	NR	NR	NR	Others	direct	x	lab	no/na	yes	above	density	reduced	2	Genetic diversity was assessed in a common garden across genetic backgrounds.	2007	Evolution		
23	<i>Galium aparine</i>	Brassicaceae	selfing	annual	autochthonous	neutral	8	100	NR	NR	NR	Within vs. among	direct	x	lab	no/na	yes	above	density	reduced	3	Genetic diversity was assessed in a common garden across genetic backgrounds.	2007	Evolution		
24	<i>Phytolacca americana</i>	Phytolaccaceae	mixed	annual	autochthonous	neutral	10	6	local	NR	NR	Others	direct	x	lab	no/na	yes	above	density	reduced	3	Genetic diversity was assessed in a common garden across genetic backgrounds.	2007	Evolution		
25	<i>Arabidopsis thaliana</i>	Brassicaceae	selfing	annual	autochthonous	neutral	11	100	NR	NR	NR	Others	direct	x	lab	no/na	yes	above	density	reduced	6	Genetic diversity was assessed in a common garden across genetic backgrounds.	2007	Evolution		

Supplementary Table 1 (continued)

Number	Species studied	Bacterial family	Metabarcoding system	Host type	Seed dispersal mode	Number of genetic clones	Number of genetic lineages	Scale of sampling	Genetic structure	Habitat	Microclimate	Diversity index	Taxonomic diversity		Macrodiversity		Total number of taxa	Geographic area covered (km <sup>2</sup> )	High-fidelity data availability	Managerial relevance	Year	Journal
													Species richness	Evenness	Number of taxa	Number of species						
26	<i>Chlorella vulgaris</i>	Rhodospirillaceae	microarray	perennial	autonomous	neutral	15	local	assorted	wetland meadows and scrubland	high to high	Shannon	1	1	1	1	1	1	1	neutral	2007	New Phytologist
27	<i>Aspergillus</i>	Trichocomaceae	microarray	perennial	autonomous	neutral	7	local	assorted	wetland meadows and scrubland	high to high	Shannon	1	1	1	1	1	1	1	neutral	2007	New Phytologist
28	<i>Sclerotinia sclerotiorum</i>	Sclerotiniaceae	microarray	perennial	autonomous	neutral	21	local	assorted	dry to dry	high to high	Shannon	1	1	1	1	1	1	1	neutral	2008	Ecology Letters
29	<i>Aspergillus</i>	Trichocomaceae	microarray	perennial	autonomous	neutral	22	local	assorted	dry to dry	high to high	Shannon	1	1	1	1	1	1	1	neutral	2008	Journal of Ecology
30	<i>Aspergillus</i>	Trichocomaceae	microarray	perennial	autonomous	neutral	14	local	assorted	dry to dry	high to high	Shannon	1	1	1	1	1	1	1	neutral	2008	New Phytologist
31	<i>Aspergillus</i>	Trichocomaceae	microarray	perennial	autonomous	neutral	8	local	assorted	dry to dry	high to high	Shannon	1	1	1	1	1	1	1	neutral	2009	American Journal of Botany
32	<i>Coleophora</i>	Gelechiaceae	microarray	perennial	autonomous	neutral	8	local	assorted	dry to dry	high to high	Shannon	1	1	1	1	1	1	1	neutral	2009	New Phytologist
33	<i>Aspergillus</i>	Trichocomaceae	microarray	perennial	autonomous	neutral	2	local	assorted	dry to dry	high to high	Shannon	1	1	1	1	1	1	1	neutral	2010	New Phytologist
34	<i>Aspergillus</i>	Trichocomaceae	microarray	perennial	autonomous	neutral	8	local	assorted	dry to dry	high to high	Shannon	1	1	1	1	1	1	1	neutral	2010	Journal of Ecology
35	<i>Aspergillus</i>	Trichocomaceae	microarray	perennial	autonomous	neutral	8	local	assorted	dry to dry	high to high	Shannon	1	1	1	1	1	1	1	neutral	2010	Journal of Ecology
36	<i>Aspergillus</i>	Trichocomaceae	microarray	perennial	autonomous	neutral	8	local	assorted	dry to dry	high to high	Shannon	1	1	1	1	1	1	1	neutral	2010	Journal of Ecology
37	<i>Aspergillus</i>	Trichocomaceae	microarray	perennial	autonomous	neutral	8	local	assorted	dry to dry	high to high	Shannon	1	1	1	1	1	1	1	neutral	2010	New Phytologist
38	<i>Aspergillus</i>	Trichocomaceae	microarray	perennial	autonomous	neutral	23	local	assorted	dry to dry	high to high	Shannon	1	1	1	1	1	1	1	neutral	2010	Journal of Ecology
39	<i>Aspergillus</i>	Trichocomaceae	microarray	perennial	autonomous	neutral	4	local	assorted	dry to dry	high to high	Shannon	1	1	1	1	1	1	1	neutral	2011	Journal of Ecology
40	<i>Aspergillus</i>	Trichocomaceae	microarray	perennial	autonomous	neutral	3	local	assorted	dry to dry	high to high	Shannon	1	1	1	1	1	1	1	neutral	2011	Journal of Ecology
41	<i>Aspergillus</i>	Trichocomaceae	microarray	perennial	autonomous	neutral	8	local	assorted	dry to dry	high to high	Shannon	1	1	1	1	1	1	1	neutral	2011	Journal of Ecology
42	<i>Aspergillus</i>	Trichocomaceae	microarray	perennial	autonomous	neutral	2	local	assorted	dry to dry	high to high	Shannon	1	1	1	1	1	1	1	neutral	2012	Journal of Ecology
43	<i>Aspergillus</i>	Trichocomaceae	microarray	perennial	autonomous	neutral	5	local	assorted	dry to dry	high to high	Shannon	1	1	1	1	1	1	1	neutral	2012	Journal of Ecology
44	<i>Aspergillus</i>	Trichocomaceae	microarray	perennial	autonomous	neutral	6	local	assorted	dry to dry	high to high	Shannon	1	1	1	1	1	1	1	neutral	2012	Journal of Ecology
45	<i>Aspergillus</i>	Trichocomaceae	microarray	perennial	autonomous	neutral	9	local	assorted	dry to dry	high to high	Shannon	1	1	1	1	1	1	1	neutral	2012	Journal of Ecology
46	<i>Aspergillus</i>	Trichocomaceae	microarray	perennial	autonomous	neutral	4	local	assorted	dry to dry	high to high	Shannon	1	1	1	1	1	1	1	neutral	2013	Journal of Ecology
47	<i>Aspergillus</i>	Trichocomaceae	microarray	perennial	autonomous	neutral	4	local	assorted	dry to dry	high to high	Shannon	1	1	1	1	1	1	1	neutral	2013	Journal of Ecology
48	<i>Aspergillus</i>	Trichocomaceae	microarray	perennial	autonomous	neutral	27	local	assorted	dry to dry	high to high	Shannon	1	1	1	1	1	1	1	neutral	2013	Journal of Ecology
49	<i>Aspergillus</i>	Trichocomaceae	microarray	perennial	autonomous	neutral	20	local	assorted	dry to dry	high to high	Shannon	1	1	1	1	1	1	1	neutral	2013	Journal of Ecology
50	<i>Aspergillus</i>	Trichocomaceae	microarray	perennial	autonomous	neutral	8	local	assorted	dry to dry	high to high	Shannon	1	1	1	1	1	1	1	neutral	2014	Journal of Ecology



**Supplementary Table 2: Summary of the list of different traits used in GxG interactions studies listed in the survey.** The list of species studied in the 77 experiments listed in the first two columns. The next four columns describe the measured trait categories which have been divided into root, shoot, life history and seed production related traits. The following two columns indicate the reference name and the year of publication. Next four columns detail the total number of traits studied in each trait category within the experiments. Following this, the total number of traits studied in each experiment is listed in the next column. Lastly, extended phenotypes, if recorded, are listed in the last column.

Number	Species studied	Measured traits				ref	year	number of traits				Tot traits	Extended phenotype
		root related traits	shoot related traits	life history traits	seed production related traits			root related traits	shoot related traits	life history traits	seed production related traits		
1	<i>Arabidopsis thaliana</i>		leaf area			Goodnight <i>et al</i>	1985	1				1	
2	<i>Pyralocaea americana</i>	dry biomass	plant height, dry biomass, number of true leaves	seedling germination	presence of buds, flowers, fruits	Willson <i>et al</i>	1987	1	3	1	3	8	
3	<i>Abutilon theophrasti</i>	dry biomass	leaf number, dry biomass	seedling germination, flowering time		Willson <i>et al</i>	1987	1	2	2		5	
4	<i>Solanum mauritanicum</i>	dry biomass	length of longest leaf, dry biomass	seedling germination		Willson <i>et al</i>	1987	1	2	1		4	
5	<i>Lycopersicon lycopersicum</i>	dry biomass	plant height	seedling germination	reproductive status	Willson <i>et al</i>	1987	1	1	1	1	4	
6	<i>Veronica peregrina</i>	dry biomass	plant height, dry biomass	seedling germination, survival, days to flowering	number of capsules per plant, total seed production	Linhart	1988	1	2	3	2	8	
7	<i>Arabidopsis thaliana</i>	whole plant weight	whole plant weight			Griffing	1989	1				1	
8	<i>Plantago lanceolata</i>		dry biomass		length of seeds, biomass of reproductive structures, no of seeds	Tonsor	1989		1		3	4	
9	<i>Ambrosia dumosa</i>	root elongation pattern				Mahall and Callaway	1991	1				1	
10	<i>Larrea tridentata</i>	root elongation pattern				Mahall and Callaway	1991	1				1	
11	<i>Impatiens capensis</i>	dry biomass	dry biomass	seed biomass		Argyres and Schmitt	1992	1	1	1		3	
12	<i>Impatiens capensis</i>		height, diameter of node, total number of nodes, dry biomass, herbivory			Burt and Bell	1992		5			5	
13	<i>Polygonum pensylvanicum</i>		height, leaf area, stem diameter, dry biomass	timing of reproductive onset	biomass of flowers, fruits, and subtending structural parts	Thomas and Bazazz	1993		4	1	1	6	
14	<i>Ambrosia dumosa</i>	root length				Mahall and Callaway	1996	1				1	
15	<i>Arabidopsis thaliana</i>		dry biomass		seed number, number of fruits	Andalo <i>et al</i>	2001		1		2	3	
16	<i>Allium vineale</i>	fresh mass of new bulbils and offsets	leaf height			Ronsheim and Anderson	2001	1	1			2	
17	<i>Cakile edentula</i>		height, stem weight		number of flowers and fruits	Donohue 2003	2003		2		2	4	
18	<i>Potentilla anserina</i>		number of ramets, number of leaves, dry biomass		biomass of flowers (including flower buds)	Rautalainen <i>et al</i>	2004		3		1	4	
19	<i>Arabidopsis thaliana</i>		dry biomass		siliqua number	Cahill <i>et al</i>	2005		1		1	2	
20	<i>Solidago altissima</i>		dry biomass, arthropod, herbivore and predator richness and abundance			Crutsinger <i>et al</i>	2006		1			1	EP:arthropod abundance
21	<i>Trifolium repens</i>	root length, biomass	shoot biomass			Falik <i>et al</i>	2006	2	1			3	
22	<i>Arabidopsis thaliana</i>	total leaf number		days to flowering		Botto and Coluccio	2007	1		1		2	
23	<i>Cakile edentula</i>	dry biomass of pot	dry biomass	biomass of reproductive structures (seed, fruits, and supporting stems and pedicels)		Dudley and File	2007	1	1	1		3	
24	<i>Plantago erecta</i>		dry biomass	seed mortality, seedling emergence		Espeland and Rice	2007		1	2		3	
25	<i>Arabidopsis thaliana</i>		height, leaf number	bolting time	Flower number, bud number, siliqua number	Mutic and Wolf	2007		2	1	3	6	
26	<i>Glechoma hederacea</i>	root distribution pattern				Semchenko <i>et al</i>	2007	1				1	
27	<i>Fragaria vesca</i>	root distribution pattern				Semchenko <i>et al</i>	2007	1				1	
28	<i>Solidago altissima</i>		stem density			Crutsinger <i>et al</i>	2008		1			1	EP:richness, cover and biomass of native and exotic colonists
29	<i>Viola tricolor</i>			<i>in vitro</i> pollen tube growth		Lankinen	2008			1		1	
30	<i>Arabidopsis thaliana</i>	biomass (in vitro), SRL, root proliferation	biomass (in vitro), crown size, SLA	height:base ratio, root:shoot biomass	siliqua number (GH)	Masclaux <i>et al</i>	2009	3	3	2	1	9	
31	<i>Impatiens pallida</i>	dry biomass of pot	Node height, total plant height, and branching, dry biomass		dry biomass of reproductive structures	Murphy and Dudley	2009	1	4		1	6	
32	<i>Cakile edeuntula</i>	dry biomass of pot	hypocotyl height, number of leaves, dry biomass of stem	number of cotyledons, cotyledon biomass		Bhatt <i>et al</i>	2010	1	3	2		6	
33	<i>Arabidopsis thaliana</i>		number of lateral roots, length of primary roots			Biedrzycki <i>et al</i>	2010	2				2	
34	<i>Arabidopsis thaliana</i>		number of lateral roots, length of primary roots, longest lateral root	hypocotyl length		Biedrzycki <i>et al</i>	2010		3	1		4	
35	<i>Abutilon theophrasti</i>	root distribution pattern				Cahill <i>et al</i>	2010	1				1	
36	<i>Arabidopsis thaliana</i>		dry biomass	survival, insect biomass		Kotowska <i>et al</i>	2010		1	1		2	EP:insect biomass
37	<i>Festuca pallens</i>		total leaf number, number of dead and green leaves, dry biomass	germination time, seed biomass, relative genome size		Smarda <i>et al</i>	2010		3	3		6	
38	<i>Arabidopsis thaliana</i>		rosette diameter	flowering time, height of inflorescence, the number of axillary branches differentiated	number of fruit produced	Willis <i>et al</i>	2010		1	3	1	5	

Number	Species studied	Measured traits				ref	year	number of traits				TOT traits	Extended phenotype
		root related traits	shoot related traits	life history traits	seed production related traits			root related traits	shoot related traits	life history traits	seed production related traits		
39	<i>Arabidopsis thaliana</i>		dry biomass, leaf number	inflorescence height	silique number	Aguilera <i>et al</i>	2011		2	1	1	4	
40	<i>Ipomea hederacea</i>	dry biomass of pot	mean plant height, height inequality in pot		seed yield of pot	Biernaskie	2011	1	2		1	4	
41	<i>Andropogon gerardii</i>	root length, dry biomass	dry biomass			Markham and Halwas	2011	2	1			3	
42	<i>Arabidopsis thaliana</i>	root exudate profile				Badri <i>et al</i>	2012	1				1	EP:exudate profile
43	<i>Taraxacum officinale</i>		total leaf area, biomass		seed production	Drummond and Vellend	2012		2		1	3	
44	<i>Ambrosia artemisiifolia</i>	biomass, mycorrhiza quantification	dry biomass, N accumulation, susceptibility to pathogen attack			File <i>et al</i>	2012	2	3			5	EP:mycorrhiza quantified
45	<i>Lupinus angustifolius</i>	root length and dry biomass	crown size, height, diameter, leaf area, dry biomass	seedling height		Milla <i>et al</i>	2012	2	5	1		8	
46	<i>Arabidopsis thaliana</i>			height of longest inflorescence at the onset of silique maturation	seed weight	Creissen <i>et al</i>	2013			1	1	2	
47	<i>Arabidopsis thaliana</i>		rosette diameter	height of longest inflorescence at the onset of silique maturation	seed weight	Creissen <i>et al</i>	2013			1	1	3	
48	<i>Artemisia tridentata</i>		herbivory damage			Karban <i>et al</i>	2013					0	EP:herbivory damage
49	<i>Arabidopsis thaliana</i>	total biomass of pot	total biomass of pot			Latzel <i>et al</i>	2013	1	1			2	
50	<i>Sedum album</i>		number of shoots	survival	number of fruits, panicles	Anderson	2014		1	1	1	2	4
51	<i>Arabidopsis thaliana</i>	root length, surface area, volume and diameter	rosette diameter, stem height, dry biomass		number of siliques, avg no seeds per silique, avg. no. seeds per plant	Bukowski and Petermann	2014	4	3		2	9	
52	<i>Arabidopsis thaliana</i>		leaf position		seed yield	Crepy and Casal	2014		1		1	2	
53	<i>Arabidopsis thaliana</i>		leaf position		seed yield	Crepy and Casal	2014		1		1	2	
54	<i>Phragmites australis</i>	dry biomass, N accumulation	dry biomass, N accumulation			Tomimatsu <i>et al</i>	2014	2	2			4	
55	<i>Trifolium pratense</i> ( 2X and 4X populations)	mycorrhizal colonization, bacterial, AM and non AM fungal richness in soil	dry biomass			Wagg <i>et al</i>	2014	1	1			2	EP:fungal/bacterial richness in soil
56	<i>Pseudoroegneria spicata</i>	dry biomass, total biomass	total biomass of pot			Yang <i>et al</i>	2015	1	1			2	
57	<i>Pseudoroegneria spicata</i>	root elongation rate				Yang <i>et al</i>	2015	1				1	
58	<i>Polygonum cespitosum</i>		dry biomass		dry biomass mature achenes, immature achenes, inflorescences, and reproductive support tissues	Corliss and Sultan	2016		1		1	2	
59	<i>Medicago minima</i>		dry biomass	survival		Ehlers <i>et al</i>	2016		1	1		2	
60	<i>Medicago rigidula</i>		dry biomass	survival		Ehlers <i>et al</i>	2016		1	1		2	
61	<i>Trifolium repens</i>		ramet number and weight, length of stolon, internode, petiole, leaf size, dry biomass			Huber <i>et al</i>	2016					8	
62	<i>Arabidopsis thaliana</i>	root allocation	lateral root number			Palmer <i>et al</i>	2016	1	7			8	
63	<i>Baccharis salicifolia</i>	dry biomass, mycorrhizal abundance	dry biomass	insect abundance		Abdala-Roberts <i>et al</i>	2017	2	1			3	EP:mycorrhizal and insect abundance
64	<i>Trifolium fucatum</i>	root transcriptomics	shoot biomass			Bowsher <i>et al</i>	2017	1	1			2	EP: root transcriptome analysis
65	<i>Xanthium italicum</i>	root length, number, angle, biomass	shoot number, length, angle, weight			El-Gawad <i>et al</i>	2017	4	4			8	
66	<i>Arabidopsis thaliana</i>		leaf number, area, leaf biomass, rosette size, plant height, tiller number		seed biomass, silique number	Jie <i>et al</i>	2017	1	7		2	10	
67	<i>Deschampsia cespitosa</i>	specific root length	length of the longest leaf, dry biomass, C and N content			Semchenko <i>et al</i>	2017	1	3			4	
68	<i>Polygonum persicaria</i>		height, dry biomass	flowering time	number of achenes, biomass of mature and immature achenes, flowers and peduncles	Baker <i>et al</i>	2018		2	1	3	6	
69	<i>Arabidopsis thaliana</i>		rosette diameter, stem height, dry biomass		number of siliques, weight	Bukowski <i>et al</i>	2018		3		2	5	
70	<i>Phalaris arundinacea</i>	dry biomass	stem height, tiller number, dry biomass			Collins <i>et al</i>	2018	1	3			4	
71	<i>Arabidopsis thaliana</i>	dry biomass	rosette area, leaf area, plant height and dry biomass		seed biomass	Li and Xu	2018	1	4		1	6	
72	<i>Moricandia moricandioides</i>		height, dry biomass	advertising effort(petal mass/tot biomass)	flower number, petal biomass	Torices <i>et al</i>	2018		2	1	2	5	
73	<i>Arabidopsis thaliana</i>			flowering time	number of siliques	Wuest and Niklaus	2018		1	1	1	2	
74	<i>Arabidopsis thaliana</i>		dry biomass, disease symptoms	flowering time, flowering bolt height	number of siliques	Wuest and Niklaus	2018		2	2	1	5	
75	<i>Arabidopsis thaliana</i>			survival	number of siliques	Zhang and Tielborger	2018		1	1	1	2	
76	<i>Medicago truncatula</i>	rhizobial community diversity, relative fitness of <i>Ensifer meliloti</i> strains				Burghardt <i>et al</i>	2019					0	EP:rhizobial community
77	<i>Arabidopsis thaliana</i>			flowering time	total seed production	Fitzpatrick <i>et al</i>	2019		1	1	1	2	

## **I.D. Prevailing questions in the study of intraspecific interactions?**

As we previously mentioned, despite the importance of plant-plant interactions in both natural plant communities and crop fields, the genetic and molecular bases underlying the natural variation of plant-plant interactions are still largely unknown. This is especially true for positive GxG interactions. In addition, whether the polymorphic genes involved in positive GxG interactions have been shaped by natural selection remains an open question. The main aim of my thesis was therefore to understand the adaptive genetic bases of intraspecific positive plant-plant interactions. To this end, I addressed three main questions:

- i. What is the extent of positive GxG interactions among and within natural plant populations?**
- ii. What are the adaptive genetic bases of positive GxG interactions?**
- iii. What are the putative ecological drivers of positive GxG interactions?**

## ***II. Arabidopsis thaliana as a model species to unravel the adaptive genetic and molecular bases of plant-plant interactions?***

### **II.A. General characteristics**

*Arabidopsis thaliana* also called the mouse ear cress or wild thale, is an annual species belonging to the Brassicaceae family. *A. thaliana* was first described by Johannes Thal in 1577 in the Harz Mountains of Northern Germany. *A.thaliana* is native across Europe, North Africa and Asia and it is a naturalized introduced species in America and Australasia.

*A. thaliana* is still the flagship species in plant physiology, genetics and molecular biology. Since more than one decade, *A. thaliana* is also widely used to study the genetics of natural phenotypic variation and eco-evolutionary responses to biotic and abiotic factors (Gaut, 2012; Roux & Bergelson, 2016). Its small size, ease of cultivation, short life cycle (in greenhouse

## General introduction

conditions) and ability to self-fertilize, allows to maintain homozygous lines and to phenotype them infinitely (Weigel & Nordborg, 2005). These characteristics, combined with the small size of its genome (5 chromosomes, ~ 119 Mb), led to the complete sequencing of its genome, the first in higher plants, in 2000 (Col-0 line, The Arabidopsis Genome Initiative 2000). The reference sequence made it possible to annotate all the genes. Currently, the database TAIR 10 (The Arabidopsis Information Resource, <http://www.arabidopsis.org/>) contains 33,518 genes including 27,379 encoding proteins (Swarbreck *et al.* 2007).

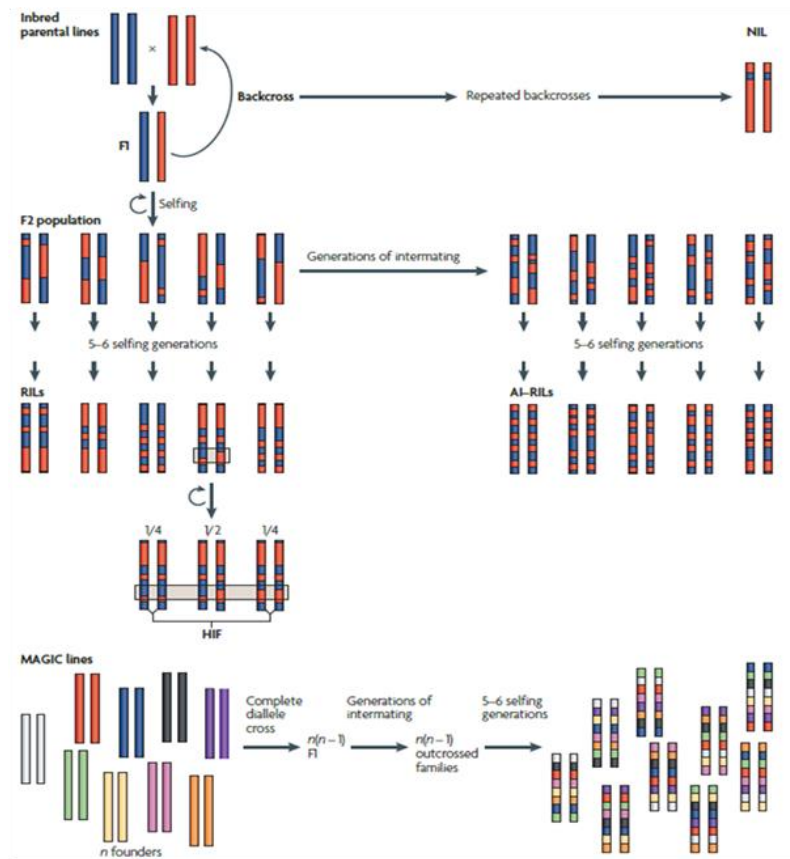
*A.thaliana* has two main types of life cycle: i) winter annuals with seeds germinating in autumn, plants overwintering as rosettes and then growing and maturing in spring, and ii) spring annuals with seeds germinating in early spring and then growing and maturing in the same season (Griffith *et al.* 2004). *A. thaliana* can produce between 10,000 and 40,000 seeds per plant. The selfing rate of 98% described in the 1980s (Abbott & Gomes 1989) has long suggested that natural populations of *A. thaliana* were predominantly monomorphic. However, since more than one decade, several studies have challenged this view. While it is true that the rate of outcrossing calculated within natural populations of *A. thaliana* is on average around 2%, this rate largely differs among natural populations and can reach up to 20% in some populations (Bomblies *et al.* 2010, Platt *et al.* 2010). Accordingly, it has been demonstrated that populations can be highly polymorphic both from a genetic and phenotypic point of view (Le Corre 2005, Platt *et al.* 2010, Kronholm *et al.* 2012, Brachi *et al.* 2013, Huard-Chauveau *et al.* 2013, Frachon *et al.* 2018).

## II.B. Genetic and genomic resources and tools available for *Arabidopsis thaliana*

Remarkable genetic resources for detecting the genetic basis of artificial phenotypic variation are publicly available in *A. thaliana* (random insertion and gene disruption: EMS mutagenesis, T-DNA and transposon mutants; specific gene disruption: artificial microRNA gene silencing, amiRNA) (Alonso & Ecker 2006, O'Malley & Ecker 2010), thus enabling functional analysis of individual genes or gene families by studying the phenotype of lines in which specific genes have been disrupted.

## General introduction

For identifying the genetic basis of natural phenotypic variation, in particular Quantitative Trait Loci (QTLs), substantial efforts have led to the creation of multiple experimental populations like  $F_2$  populations, Recombinant Inbred Lines (RILs) families, as well as Multiparent Advanced Inter-Cross Generation (MAGIC) lines (Figure 1). RILs corresponds to lines from  $F_2$  progeny that have undergone several generations of inbreeding to obtain almost complete homozygous lines representing a unique mosaic of the two parental genomes (Bazakos *et al.* 2017). These populations have become very popular because RILs can be phenotyped almost indefinitely but genotyped only once since they are quasi-homozygous lines (Savolainen *et al.* 2013, Bazakos *et al.* 2017). RIL families make it possible to have replicates of the same genotype within one experiment and / or between several environmental conditions and therefore remain the most common type of experimental population used in *A. thaliana* (Bergelson & Roux 2010).



**Figure 1: Illustration of the different populations that can be used to map genetic markers associated with natural phenotypic variation by traditional QTL mapping. RILs: Recombinant inbred lines, AI-RILs: Advanced intercross-recombinant inbred lines, HIF: Heterogeneous inbred family, MAGIC: multiparent advanced generation inter-cross lines, NIL: near-isogenic line (Bergelson & Roux 2010).**

## General introduction

In addition, extensive collections of natural accessions collected from a worldwide scale to a local scale are also available for this species. In recent years, a considerable effort has been put in place to genetically characterize these natural accessions. For instance, in order to get a better accuracy in the identification of the genetic bases associated with natural phenotypic variation (*i.e.*, obtain direct access to causal mutations), the complete sequencing of the genome of approximately 1200 worldwide natural accessions of *A. thaliana* has been produced *via* an international consortium (<http://www.1001genomes.org>).

In order to study the adaptive dynamics of *A. thaliana* at a more restricted geographical scale, our team obtained a representative picture of within population genetic variation across the genome of 217 French populations by adopting a Pool-Seq approach (Frachon *et al.* 2018, Bartoli *et al.* unpublished data). For a given population, this approach consists in extracting the DNA of each individual, then creating a bulk in an equimolar manner and sequencing this bulk (Schlötterer *et al.* 2014). These populations have been sampled in five French regions that have contrasting climates, *i.e.* Brittany (n = 11), Burgundy (n = 11), Languedoc (n = 16), North (n = 11) and Midi-Pyrénées (n = 168) (Brachi *et al.* 2013, Frachon *et al.* 2018). In addition, by sequencing the genome of 195 local natural accessions, our team revealed that the genetic diversity within a local population located in Burgundy (population TOU-A, France) can represent up to one-sixth of the total natural genetic diversity observed at the worldwide scale (Frachon *et al.* 2017).

All these resources are publicly available through many international seed stock centers. The main ones are the Nottingham Arabidopsis Stock Centre (NASC, <http://arabidopsis.info>), the Arabidopsis Biological Resource Center (ABRC, <https://abrc.osu.edu>) and the Versailles Arabidopsis Stock Center of INRA (<http://publiclines.versailles.inra.fr>). Several database platforms are also in place to provide genetic and molecular data of *A. thaliana* such as the Arabidopsis International Resource (TAIR 10, <https://www.arabidopsis.org>), the Bio-Analytic Resource for Plant Biology (BAR, <https://www.bar.utoronto.ca>) and the plant ontology database 'Planteome' (<http://planteome.org>).

## II.C. A model for studying natural variation of plant-plant interactions?

*A. thaliana* is present in a great diversity of habitats with highly variable biotic and abiotic environments on its global range (Jakob *et al.* 2002, Mitchell-Olds & Schmitt 2006, Shindo *et al.* 2007). This diversity of habitats can be observed even at a geographical scale of about a few kilometers (Brachi *et al.* 2013), and even few meters (Frachon *et al.* 2017). While *A. thaliana* was initially described as a pioneer species often found in poor or disturbed environments, rarely in competition with other species, recent reports and observations on the ground seem to prove the opposite. Indeed, during various surveys of natural populations of *A. thaliana* in France carried out by the team since 2009, *A. thaliana* has been observed in very competitive environments.

At the French scale, the 49 populations of *A. thaliana* collected from Burgundy, Brittany, Languedoc and North, have been characterized (i) phenotypically under controlled conditions for six phenological traits such as flowering time, and (ii) ecologically for 42 variables including climate, soil agronomic properties and intensity of plant-plant interactions. A phenotype-ecology correlational approach suggested that in some French regions, interspecific plant-plant interactions can be the main selective agent acting on phenological traits (Brachi *et al.* 2013).

At the regional scale, the 168 natural populations of *A. thaliana* collected from ecologically contrasted habitats in the Midi-Pyrénées region were characterized for a large range of biotic factors including microbiota as well as plant communities (Bartoli *et al.* 2018, Frachon *et al.* 2018, Frachon *et al.* 2019). The characterization of the plant communities associated with these 168 populations showed that *A. thaliana* could coexist with contrasted plant communities, both in terms of richness (min = 1, max = 28 plant species, mean = 12.1) and composition (Frachon *et al.* 2019) (Figure 2).

At the local scale, an *in-situ* resurrection experiment based on 195 accessions collected in 2002 and 2010 in the local population TOU-A located between two permanent meadows demonstrated an adaptive phenotypic evolution in less than eight generations, probably partly in response to increased competition (Frachon *et al.* 2017). In addition, by growing in a field 48 TOU-A accessions in absence and presence of four species frequently associated with *A. thaliana* in its natural plant communities (*i.e.*, *Poa annua*, *Stellaria media*, *Trifolium arvense* and *Veronica arvensis*), we showed that crossing reaction norms of competitive response (*A. thaliana*

## General introduction

performance) and effect (competitor biomass) might promote maintenance of genetic variation in a local population of *A. thaliana* and species coexistence at a fine spatial scale (Baron *et al.* 2015).



**Figure 2: Photographs of *A. thaliana* in environments with strong plant-plant interactions in the Midi-Pyrénées region. The red arrows indicate *A. thaliana* (according to Léa Frachon's thesis).**

For these reasons, our natural populations of *A. thaliana* collected at complementary geographical scales over a large range of environments with varying abiotic and biotic factors shaping natural interactions constitute a marvelous resource for (i) studying GxG interactions in wild plant populations, (ii) dissecting their underlying adaptive genetic and molecular mechanisms, and (iii) identifying the ecological factors acting as selective agents.

### III. How to identify the genetic basis of adaptation?

In order to determine the adaptive potential of natural populations in the face of global environmental changes of abiotic and / or biotic origin (Bergelson & Roux 2010, Bay *et al.* 2017), one of the major challenges in evolutionary ecology is to characterize the genetic architecture of adaptation. It specifically involves addressing the following questions: (i) what is the number of genes underlying local adaptation? (ii) what is the distribution of allelic effects? (iii) what is the identity of adaptive genes and associated biological functions? (iv) do pleiotropy and epistasis contribute to the adaptive walk towards a new phenotypic optimum?

## General introduction

By taking advantage of the recent development of NGS to obtain an unprecedented number of genetic markers (including Single Nucleotide Polymorphism, SNP), four complementary approaches based on genomic data can be used to study the genetic architecture of adaptation, and more specifically to fine map the genes underlying adaptation (Table 1).

Approach	Data collected/resources required	What analysis reveals
Genetic differentiation outlier tests	Genome-wide SNPs from multiple populations	Allele frequencies for a SNP or SNPs that are differentiated across populations above what is expected from neutrality
Genetic-environment association	Genome-wide SNPs from multiple populations and environmental data for each population	Alleles at a SNP or SNPs that are associated with environmental variables over space
GWAS	Genome-wide SNPs from hundreds of individuals grown in one or multiple common gardens; phenotypes and/or fitness for each individual	Identifies SNPs that are associated with traits associated with fitness measured under field conditions
Population-specific selective sweeps	Genome-wide SNPs from at least two populations and a recombination map	DNA sequences with longer-than-expected regions of extended haplotype homozygosity, which is consistent with a recent selective sweep in one of the populations

**Table 1: Different approaches to identify the genetic basis of local adaptation (from Hoban *et al* 2016). SNP: single nucleotide polymorphism, GWAS: genome-wide association studies.**

The approaches 'genetic differentiation outlier tests' and 'population-specific selective sweeps' seek to identify genomic regions whose patterns of diversity and selection deviate from those expected under neutral selective processes. Although widely used, both methods provide a list of candidate genes that are often difficult to link to adaptive phenotypic traits or potential selective agents. To overcome this drawback, statistical methods to identify genetic polymorphisms along the genome associated with either phenotypic traits (traditional QTL mapping and Genome-Wide Association mapping), or ecological variables (Genome-Environment Association) have been developed.

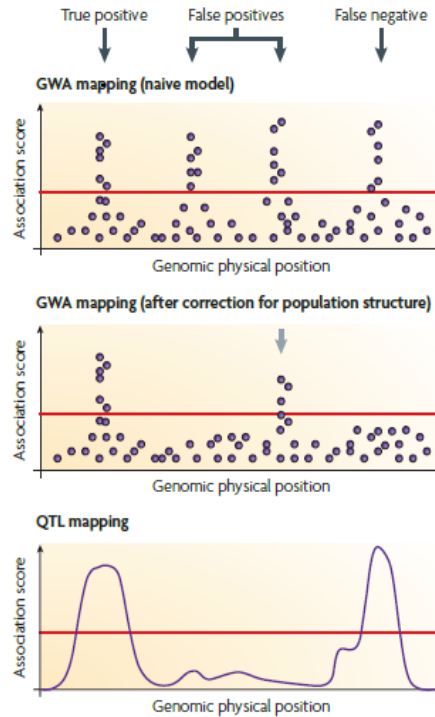
### III.A. Genome wide association (GWA) mapping

Genome Wide Association (GWA) mapping aims to identify across the genome, genetic polymorphisms associated with phenotypic variation measured in a set of natural genotypes (Bush and Moore, 2012; Read and Massey, 2014). GWA mapping was first developed for medical and pharmacological purposes (Haines *et al.* 2005, Klein *et al.* 2005, Cooper *et al.* 2008, Bergelson & Roux 2010) and since more than a decade, it has also been used in plants (Aranzana *et al.* 2005, Zhao *et al.* 2007, Atwell *et al.* 2010). GWA mapping is a powerful tool that relies on the short linkage disequilibrium (LD) observed in natural populations, resulting from tens thousands of generations of recombination. LD is an estimate of the relationship between two genetic polymorphisms and the physical distance between them (Bush & Moore 2012). For a more precise mapping resolution, it is better to have a short LD and a genetically very diverse population (Sauvage *et al.* 2014). Typically, estimates of LD decay in *A. thaliana* is between 5 and 10kb, but can largely vary among geographical regions (Horton *et al.* 2012). For instance, LD decay is longer in regions recently colonized by *A. thaliana* such as Northern Europe and USA. On the other hand, LD decay can be shorter than 2kb in highly polymorphic populations of *A. thaliana*, such as in the TOU-A population in France (Frachon *et al.* 2017).

However, GWA has two important disadvantages, that is, the presence of false positives and genetic and/or allelic heterogeneity. The production of false positives corresponds to a false genotype-phenotype association due to the effect of the species demographical history. The effect of population structure in GWA mapping is particularly important in *A. thaliana* at the European scale, due to the colonization of Northern regions from several glacial refugia located around the Mediterranean Sea during the last 10,000 years (The 1000 Genomes Consortium). To reduce the rate of false-positives, mixed models including a kinship matrix can be used to control for confounding by population structure (Kang *et al.* 2010). However, this statistical correction can lead to an increase of the rate of false negatives, which are causative genetic markers that are lost after correcting for population structure (Figure 3, Bergelson & Roux 2010). Therefore, to limit the rate of false positives and false negatives, GWA mapping can be combined with traditional linkage mapping (based on F2 populations, RILs...), which is prone for identifying rare alleles and is not subject to the effect of population structure (Bergelson & Roux 2010). Nonetheless, these combined approaches remain time consuming due to the requested phenotyping of thousands

## General introduction

experimental lines (Brachi *et al.* 2010). An alternative approach to limit the drawbacks of GWA mapping performed at a worldwide scale is to work at a small geographical scale, thereby limiting the effect of demographic history (Bergelson & Roux 2010).



**Figure 3: Illustration of false positives and false negatives in GWA mapping studies (Bergelson & Roux 2010).**

The second major disadvantage of GWA is the presence of genetic and/or allelic heterogeneity. This happens when the same observed phenotypic value within a mapping population results from different QTLs or different alleles of the same QTL (Figure 3, Bergelson & Roux 2010). Genetic and/or allelic heterogeneity largely diminishes the statistical power of GWA mapping, since it increases the level of asymmetry between intra-allelic variances to a given genetic marker (Figure 4). To solve this problem, it is also recommended to work at a small geographical scale in order to limit the number of rare alleles, while maintaining a relative high genetic diversity in comparison to the worldwide scale (Bergelson & Roux 2010).

## General introduction

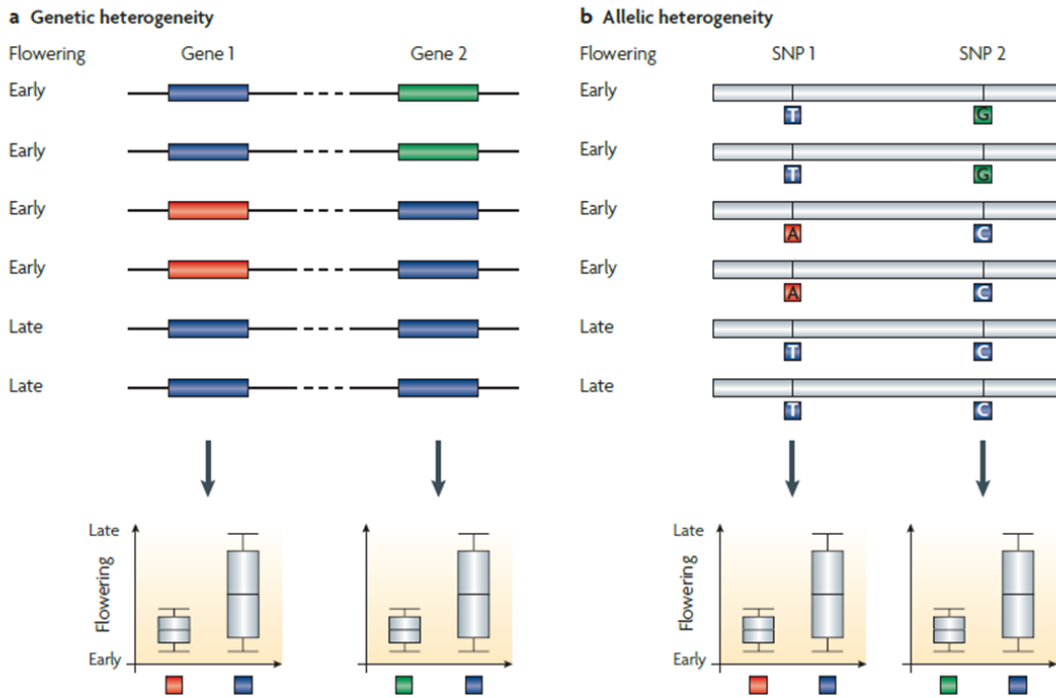


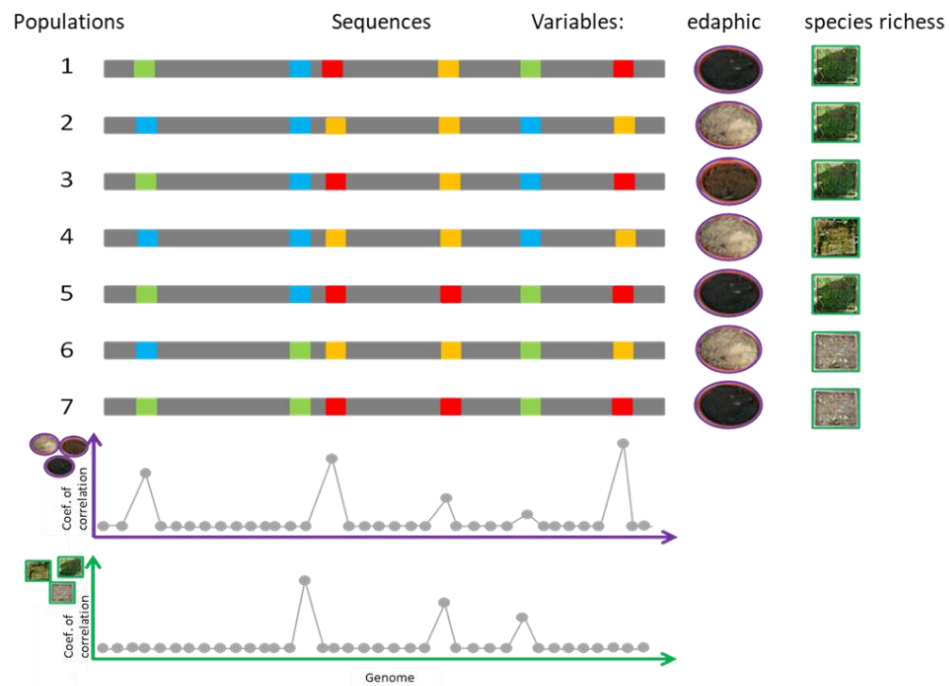
Figure 4: Schematic representation of the effect of genetic and allelic heterogeneity on QTL detection in GWA mapping in the case of flowering time (Bergelson & Roux 2010).

### III.B. Genome-Environment Association (GEA)

Genome-Environment Association (GEA) analyzes are based on the effect of spatial selective ecological gradients on the genomic variation of a given species (Lasky *et al.* 2012). Indeed, a heterogeneous environment varying both at the abiotic and biotic scales leads to different local phenotypic optima, which will result in a spatial differentiation of the genetic variants underlying the phenotypes involved in the response to selective agents. This type of analysis therefore not only makes it possible to identify genes potentially involved in adaptation but also to describe the ecological factors responsible for their genetic divergence between populations (Pluess *et al.* 2016, Figure 5). Initially carried out by individual-centered approaches where only one individual per population was genomically characterized, GEA-type analyzes were subsequently performed using population-based approaches to benefit from information provided by intra-population genetic variation (Frachon *et al.* 2018).

## General introduction

In *A. thaliana*, one of the first GEA-type studies along the genome in a wild species was conducted using an individual-centric approach (Hancock *et al.* 2011). To carry out this study, the authors relied on 948 European accessions of the *A.thaliana* genotyped for 215k SNPs and characterized for 13 climatic variables including extremes and seasonality of temperature and precipitation, photosynthetically active radiation (PAR), relative humidity, season lengths, and aridity. To identify genomic regions associated with climatic variation, the authors used a partial Mantel test based on the calculation of Spearman's correlation coefficient between a given SNP and a climatic variable, while controlling for the effect of demographic history. These analyzes made it possible to draw up a genomic map of climate adaptation in *A. thaliana*. In particular, the SNPs most strongly correlated with climate were significantly enriched in genetic variants corresponding to amino acid changes. In addition, using this approach, the authors were able to successfully predict the relative fitness of each accession in a particular climatic environment. Altogether, this GEA-type individual-centric approach has made it possible to identify adaptive loci in geographically diverse *A.thaliana* accessions.



**Figure 5: Principle of the Genome-Environment Association (GEA) analysis.** Genomes are represented for seven populations. On the top, the characterization of an edaphic variable and descriptors of plant communities is indicated for each population. At the bottom of the diagram, the correlation coefficients between genetic variation and ecological variation (violet: edaphic variable, green: species richness) are plotted against the position of polymorphic genetic markers along the genome (from Léa Frachon's thesis).

## General introduction

Although very powerful, individual-centric approaches neglect genetic variation within populations. It is important to consider intra-population genetic variation to obtain a better estimate of the adaptive potential of natural populations. These population-centric approaches have been little used until now because sequencing of several individuals per population can still be very expensive. To overcome this problem, an alternative has been proposed by adopting the Pool-Seq approach. Therefore, a representative picture of within-population genetic variation across the genome can be obtained by such an approach (Frachon *et al.* 2018). Whole-genome scan association with ecological variables can be performed then by using various statistical models (Coop *et al.* 2010), including Bayesian hierarchical models that takes into account the demographic history of the species by estimating a covariance matrix of the population allelic frequencies between populations from all available genetic markers (Gautier 2015). Using this methodology, a GEA analysis was performed in our team to investigate the genetic basis of adaptation to six climate variables in the 168 natural populations collected in the Midi-Pyrénées region (Frachon *et al.* 2018). The obtained results gave evidence that climate is also an important driver for adaptive genomic variation even at the regional scale. More recently, this approach has been extended to biotic factors wherein the authors were able to fine map adaptive genomic regions of *A. thaliana* associated with plant community descriptors (Frachon *et al.* 2019). Interestingly, the adaptive loci associated with species abundance were highly dependent on the identity of the neighboring species suggesting a high degree of biotic specialization of *A. thaliana* to members of its plant interaction network. Moreover, the identification of adaptive loci associated with alpha-diversity and composition of plant communities supported the ability of *A. thaliana* to interact simultaneously with multiple plant neighbors, which in turn can help to understand the role of community-wide selection.

## IV. Outline of the thesis

By adopting an interdisciplinary approach between population ecology, genomics and association genetics, I was interested in finding the adaptive genetic bases of intraspecific positive interactions in *Arabidopsis thaliana*.

## General introduction

In the first chapter of my thesis, I will detail an experiment conducted to study the natural variation of GxG interactions among whole-genome sequenced wild populations of *A. thaliana* from the Midi-Pyrénées region. To study the variation in the degree of overyielding (intergenotype performance > intragenotype performance) / kin cooperation (intragenotype performance > intergenotype performance) among natural populations, a field experiment was set up using 52 natural populations of *A. thaliana* collected in contrasted abiotic and biotic habitats. Three genotypes from each population were grown either alone, with the same genotype, or with another genotype from the same population. For all accessions, aboveground dry biomass was estimated at the end of the experiment. I will detail GWA mapping results describing the genetic architecture underlying natural variation in overyielding / kin cooperation among the 52 populations phenotyped during the field experiment. I will also discuss the results obtained for the investigation of the type and strength of selection acting on QTLs associated with the observed variation of these two types of positive interactions. Finally, I will discuss the major results obtained for the identification of putative selective agents driving this variation of overyielding / kin cooperation among natural populations.

The second chapter of my thesis will detail an experimental study that was conducted by Cyril Libourel (a former PhD student in the team) in order to study the intra-population genetic variation of intraspecific interactions, in particular positive interactions. To do so, a greenhouse experiment was set up by growing 192 target accessions of a highly polymorphic French local population (i.e. TOU-A population) alone or in presence of each of three other partner accessions (tester accessions) from the same population. Aboveground vegetative growth of the target and partner plants was approximated by maximal rosette diameter. As the genome of the 195 accessions used in this study has been sequenced (Frachon *et al.* 2017), I will describe the results obtained from a GWA mapping approach that I adopted to detect QTLs associated with positive interactions within this local population. Based on these QTLs, I was able to test three complementary hypotheses for explaining reciprocal helping (*i.e.* cooperation) between some accessions in this local population, i.e. (i) kin selection theory, (ii) Greenbeard effect hypothesis, and (iii) compatibility gene hypothesis.

In a last chapter, I will discuss the main conclusions based on the experimental results obtained from the observation of variation of positive interactions among and within natural

## General introduction

populations of *A. thaliana*. Finally, I will discuss future perspectives and avenues that could be considered to finally dissect the genetic and molecular mechanisms underlying positive interactions in *A. thaliana*.

## V. References

- 1001 genomes project. (2016). 1,135 genomes reveal the global pattern of polymorphism in *Arabidopsis thaliana*. *Cell*, 166: 481–491.
- Abbott, R. J., & Gomes, M. F. (1989). Population genetic structure and outcrossing rate of *Arabidopsis thaliana* (L) Heynh. *Heredity*, 62(3), 411–418. <https://doi.org/10.1038/hdy.1989.56>
- Alonso, J. M., & Ecker, J. R. (2006). Moving forward in reverse: Genetic technologies to enable genome-wide phenomic screens in Arabidopsis. *Nature Reviews Genetics*. <https://doi.org/10.1038/nrg1893>
- Aranzana, M. J., Kim, S., Zhao, K., Bakker, E., Horton, M., Jakob, K., Lister, C., Molitor, J., Shindo, C., Tang, C., Toomajjan, C., Traw, B., Zheng, H., Berelson, J., Dean, C., Marjoram, P., & Nordborg, M. (2005). Genome-Wide Association mapping in *Arabidopsis* identifies previously known flowering time and pathogen resistance genes. *PLoS Genetics*, 1(5), e60. <https://doi.org/10.1371/journal.pgen.0010060>
- Atwell, S., Huang, Y.S., Vilhjálmsson, B.J., Willems, G., Horton, M., Li, Y., Meng, D., Platt, A., Tarone, A.M., Hu, T.T., Jiang, R., Mulyati, N.W., Zhang, X., Amer, M.A., Baxter, I., Brachi, B., Chory, J., Dean, C., Debieu, M., de Meaux, J., Ecker, J.R., Faure, N., Kniskern, J.M., Jones, J.D., Michael, T., Nemri, A., Roux, F., Salt, D.E., Tang, C., Todesco, M., Traw, M.B., Weigel, D., Marjoram, P., Borevitz, J.O., Bergelson, J., & Nordborg, M. (2010). Genome-wide association study of 107 phenotypes in *Arabidopsis thaliana* inbred lines. *Nature*. 465 (7296), 627–31
- Baron, E., Richirt, J., Villoutreix, R., Amsellem, L., & Roux, F. (2015). The genetics of intra- and interspecific competitive response and effect in a local population of an annual plant species. *Functional Ecology*, 29(10), 1361–1370. <https://doi.org/10.1111/1365-2435.12436>
- Bartoli, C., Frachon, L., Barret, M., Rigal, M., Huard-Chauveau, C., Mayjonade, B., Zanchetta, C., Bouchez, O., Roby, D., Carrere, S., & Roux, F. (2018). *In situ* relationships between microbiota and potential pathobiota in *Arabidopsis thaliana*. *ISME Journal*, 12(8), 2024–2038. <https://doi.org/10.1038/s41396-018-0152-7>
- Bay, R. A., Rose, N., Barrett, R., Bernatchez, L., Ghalambor, C. K., Lasky, J. R., Brem, R.B., Palumbi, S.R., & Ralph, P. (2017). Predicting responses to contemporary environmental change using evolutionary response architectures. *The American Naturalist*, 189(5), 463–473. <https://doi.org/10.1086/691233>
- Bazakos, C., Hanemian, M., Trontin, C., Jiménez-Gómez, J. M., & Loudet, O. (2017). New Strategies and Tools in Quantitative Genetics: How to go from the phenotype to the genotype. *Annual Review of Plant Biology*, 68(1), 435–455. <https://doi.org/10.1146/annurev-arplant-042916-040820>
- Bergelson, J., & Roux, F. (2010). Towards identifying genes underlying ecologically relevant traits in *Arabidopsis thaliana*. *Nature Reviews Genetics*. <https://doi.org/10.1038/nrg2896>
- Bertness, M. D., & Callaway, R. (1994). Positive interactions in communities. *Trends in Ecology and Evolution*, 9(5), 187–191. [https://doi.org/10.1016/0169-5347\(94\)90087-6](https://doi.org/10.1016/0169-5347(94)90087-6)

## General introduction

- Bomblies, K., Yant, L., Laitinen, R. A., Kim, S.-T., Hollister, J. D., Warthmann, N., Fitz, J., & Weigel, D. (2010). Local-scale patterns of genetic variability, outcrossing, and spatial structure in natural stands of *Arabidopsis thaliana*. *PLoS Genetics*, 6(3), e1000890. <https://doi.org/10.1371/journal.pgen.1000890>
- Brachi, B., Faure, N., Bergelson, J., Cuguen, J., & Roux, F. (2013). Genome-wide association mapping of flowering time in *Arabidopsis thaliana* in nature: genetics for underlying components and reaction norms across two successive years. *Acta Botanica Gallica*, 160(3–4), 205–219. <https://doi.org/10.1080/12538078.2013.807302>
- Brachi, B., Faure, N., Horton, M., Flahauw, E., Vazquez, A., Nordborg, M., Bergelson, J., Cuguen, J., & Roux, F. (2010). Linkage and association mapping of *Arabidopsis thaliana* flowering time in nature. *PLoS Genetics*, 6(5), 40. <https://doi.org/10.1371/journal.pgen.1000940>
- Brachi, B., Villoutreix, R., Faure, N., Hautekèete, N., Piquot, Y., Pauwels, M., Roby, D., Cuguen, J., Bergelson, J., & Roux, F. (2013). Investigation of the geographical scale of adaptive phenological variation and its underlying genetics in *Arabidopsis thaliana*. *Molecular Ecology*, 22(16), 4222–4240. <https://doi.org/10.1111/mec.12396>
- Brooker, R. W. (2006). Plant-plant interactions and environmental change. *New Phytologist*, 171(2), 271–284. <https://doi.org/10.1111/j.1469-8137.2006.01752.x>
- Brooker, R. W., & Callaghan, T. V. (1998). The balance between positive and negative plant interactions and its relationship to environmental gradients: A Model. *Oikos*, 81(1), 196. <https://doi.org/10.2307/3546481>
- Bruno, J. F., Stachowicz, J. J., & Bertness, M. D. (2003). Inclusion of facilitation into ecological theory. *Trends in Ecology and Evolution*, 18(3), 119–125. [https://doi.org/10.1016/S0169-5347\(02\)00045-9](https://doi.org/10.1016/S0169-5347(02)00045-9)
- Bush, W. S., & Moore, J. H. (2012). Chapter 11: Genome-Wide Association studies. *PLoS Computational Biology*, 8(12). <https://doi.org/10.1371/journal.pcbi.1002822>
- Callaway, R. M. (1995). Positive interactions among plants. *The Botanical Review*, 61(4), 306–349. <https://doi.org/10.1007/BF02912621>
- Chaney, L., & Baucom, R. S. (2014). The costs and benefits of tolerance to competition in *Ipomoea purpurea*, the common morning glory. *Evolution*, 68(6), 1698–1709. <https://doi.org/10.1111/evo.12383>
- Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics*, 31(1), 343–366. <https://doi.org/10.1146/annurev.ecolsys.31.1.343>
- Coop, G., Witonsky, D., Rienzo, A. Di, & Pritchard, J. K. (2010). Using environmental correlations to identify loci underlying local adaptation. *Genetics*, 185(4), 1411–23. <https://doi.org/10.1534/genetics.110.114819>
- Cooper, J. D., Smyth, D. J., Smiles, A. M., Plagnol, V., Walker, N. M., Allen, J. E., Downes, K., Barrett, J.C., Healy, B.C., Mychaleckyl, J.C., Warram, J.H., & Todd, J. A. (2008). Meta-analysis of genome-wide association study data identifies additional type 1 diabetes risk loci. *Nature Genetics*, 40(12), 1399–1401. <https://doi.org/10.1038/ng.249>
- File, A. L., Murphy, G. P., & Dudley, S. A. (2012). Fitness consequences of plants growing with siblings: Reconciling kin selection, niche partitioning and competitive ability. *Proceedings of the Royal Society B: Biological Sciences*, 279(1727), 209–218. <https://doi.org/10.1098/rspb.2011.1995>
- Frachon, L., Bartoli, C., Carrère, S., Bouchez, O., Chaubet, A., Gautier, M., Roby, D., & Roux, F. (2018). A genomic map of climate adaptation in *Arabidopsis thaliana* at a micro geographic scale. *Frontiers in Plant Science*, 9, 1–15. <https://doi.org/10.3389/fpls.2018.00967>

## General introduction

- Frachon, L., Libourel, C., Villoutreix, R., Carrère, S., Glorieux, C., Huard-Chauveau, C., Navascues, M., Gay, L., Vitalis, R., Baron, E., Amsellem, L., Bouchez, O., Vidal, M., Le Corre, V., Roby, D., Bergelson, J., & Roux, F. (2017). Intermediate degrees of synergistic pleiotropy drive adaptive evolution in ecological time. *Nature Ecology & Evolution*, 1, 1551-1561. <https://doi.org/10.1038/s41559-017-0297-1>
- Frachon, L., Mayjonade, B., Bartoli, C., Hautekèete, N. C., & Roux, F. (2019). Adaptation to plant communities across the genome of *Arabidopsis thaliana*. *Molecular Biology and Evolution*, 36(7), 1442–1456. <https://doi.org/10.1093/molbev/msz078>
- Gaut, B. (2012). *Arabidopsis thaliana* as a model for the genetics of local adaptation. *Nature Genetics*, 44(2), 115–116. <https://doi.org/10.1038/ng.1079>
- Gautier, M. (2015). Genome-wide scan for adaptive divergence and association with population-specific covariates. *Genetics*, 201(4), 1555–1579. <https://doi.org/10.1534/genetics.115.181453>
- Goldberg, D. E., & Barton, A. M. (1992). Patterns and consequences of interspecific competition in natural communities: a review of field experiments with plants. *American Naturalist*, 139(4), 771–801. <https://doi.org/10.1086/285357>
- Griffith, C., Kim, E., & Donohue, K. (2004). Life-history variation and adaptation in the historically mobile plant *Arabidopsis thaliana* (Brassicaceae) in North America. *American Journal of Botany*, 91(6), 837–849. <https://doi.org/10.3732/ajb.91.6.837>
- Gürerk, Ö., Irlenbusch, B., & Rockenbach, B. (2006). The competitive advantage of sanctioning institutions. *Science*, 312(5770), 108–111. <https://doi.org/10.1126/science.1123633>
- Haines, J. L., Hauser, M. A., Schmidt, S., Scott, W. K., Olson, L. M., Gallins, P., Spencer, k.L., Kwan, S.Y., Noureddine, M., Gilbert, J.R., Schnetz-Boutaud, N., Agarwal, A., Postel, E.A., & Pericak-Vance, M. A. (2005). Complement factor H variant increases the risk of age-related macular degeneration. *Science*, 308(5720), 419–421. <https://doi.org/10.1126/science.1110359>
- Hoban, S., Kelley, J.L., Lotterhos, K.E., Antolin, M.F., Bradburd, G., Lowry, D.B., Poss, M.L., Reed, L.K., Storfer, A., & Whitlock, M.C. (2016) Finding the genomic basis of local adaptation: pitfalls, practical solutions, and future directions. *The American Naturalist*, 188, 379–397
- Horton, M. W., Hancock, A. M., Huang, Y. S., Toomajian, C., Atwell, S., Auton, A., Mulyati, N.W., Platt, A., Sperone, Vilhjalmsson, B.J. Nordborg, M., Borevitz, J.O., & Bergelson, J (2012). Genome-wide patterns of genetic 646 variation in worldwide *Arabidopsis thaliana* accessions from the RegMap panel. *Nature Genetics*, 44(2), 212–216. <https://doi.org/10.1038/ng.1042>.Genome-wide
- Huard-Chauveau, C., Perchepied, L., Debieu, M., Rivas, S., Kroj, T., Kars, I., Bergelson, J., Roux, F., & Roby, D. (2013). An atypical kinase under balancing selection confers broad spectrum disease resistance in *Arabidopsis*. *PLoS Genetics* 9(9).doi:10.1371/journal.pgen.1003766
- Jakob, K., Goss, E. M., Araki, H., Van, T., Kreitman, M., & Bergelson, J. (2002). *Pseudomonas viridiflava* and *P. syringae* - Natural pathogens of *Arabidopsis thaliana*. *Molecular Plant-Microbe Interactions*, 15(12), 1195–1203. <https://doi.org/10.1094/MPMI.2002.15.12.1195>
- Kang, H., Sul, J., Service, S., Zaitlen, N., Kong, S.Y., Freimer, N.B., Sabatti, C., & Eskin, E. (2010). Variance component model to account for sample structure in genome-wide association studies. *Nature Genetics* 42(4),348-54

## General introduction

Keddy, P.A. (2015) Competition in plant communities. *Oxford Bibliographies in Ecology*. <https://doi.org/10.1093/obo/9780199830060-0009>

Kronholm, I., Picó, F. X., Alonso-Blanco, C., Goudet, J., & Meaux, J. de. (2012). Genetic basis of adaptation in *Arabidopsis thaliana*: Local adaptation at the seed dormancy QTL DOG1. *Evolution*, 66(7), 2287–2302. <https://doi.org/10.1111/j.1558-5646.2012.01590.x>

Le Corre, V. (2005). Variation at two flowering time genes within and among populations of *Arabidopsis thaliana*: comparison with markers and traits. *Molecular Ecology*, 14(13), 4181–4192. <https://doi.org/10.1111/j.1365-294X.2005.02722.x>

Martorell, C., & Freckleton, R. P. (2014). Testing the roles of competition, facilitation and stochasticity on community structure in a species-rich assemblage. *Journal of Ecology*, 102(1), 74–85. <https://doi.org/10.1111/1365-2745.12173>

Mitchell-Olds, T., & Schmitt, J. (2006). Genetic mechanisms and evolutionary significance of natural variation in *Arabidopsis*. *Nature*, 441(7096), 947–52.

O'Malley, R. C., & Ecker, J. R. (2010). Linking genotype to phenotype using the *Arabidopsis* unimutant collection. *The Plant Journal*, 61(6), 928–940. <https://doi.org/10.1111/j.1365-313X.2010.04119.x>

Pierik, R., Mommer, L., & Voeselek, L. A. (2013). Molecular mechanisms of plant competition: neighbour detection and response strategies. *Functional Ecology*, 27(4), 841–853. <https://doi.org/10.1111/1365-2435.12010>

Platt, A., Horton, M., Huang, Y. S., Li, Y., Anastasio, A. E., Mulyati, N. W., Agren, J., Bossdorf, O., Byers, D., Donohue, K., Dunning, M., Holub, E.B., Hudson, A., Le Corre, V., Loudet, O., Roux, F., Warthmann, N., Weigel, D., Rivero, L., Scholl, R., Nordborg, M., Bergelson, J., & Borevitz, J. O. (2010). The scale of population structure in *Arabidopsis thaliana*. *PLoS Genetics*, 6(2). <https://doi.org/10.1371/journal.pgen.1000843>

Pluess, A. R., Frank, A., Heiri, C., Lalagüe, H., Vendramin, G. G., & Oddou-Muratorio, S. (2016). Genome-environment association study suggests local adaptation to climate at the regional scale in *Fagus sylvatica*. *New Phytologist*, 210(2), 589–601. <https://doi.org/10.1111/nph.13809>

Read, T. D., & Massey, R. C. (2014). Characterizing the genetic basis of bacterial phenotypes using genome-wide association studies: a new direction for bacteriology. *Genome Medicine*, 6(11), 109. <https://doi.org/10.1186/s13073-014-0109-z>

Risch, N., & Merikangas, K. (1996). The future of genetic studies of complex human diseases. *Science* 273(5281), 1516–1517.

Roux, F., & Bergelson, J. (2016). The genetics underlying natural variation in the biotic interactions of *Arabidopsis thaliana*: The challenges of linking evolutionary genetics and community ecology. In *Current Topics in Developmental Biology* 119, 111–156.

Sauvage, C., Segura, V., Bauchet, G., Stevens, R., Do, T., Nikoloski, Z., & Causse, M. (2014). Genome-Wide Association in tomato reveals 44 candidate loci for fruit metabolic traits. *Plant Physiology*. 165(3), 1120–1132. <https://doi.org/10.1104/pp.114.241521>

Savolainen, O., Lascoux, M., & Merilä, J. (2013). Ecological genomics of local adaptation. *Nature Reviews Genetics*.14(11), 807–20. <https://doi.org/10.1038/nrg3522>

Schlötterer, C., Tobler, R., Kofler, R., & Nolte, V. (2014). Sequencing pools of individuals-mining genome-wide polymorphism data without big funding. *Nature reviews Genetics*.15(11),749–63. <https://doi.org/10.1038/nrg3803>

## General introduction

- Shindo, C., Bernasconi, G., & Hardtke, C. S. (2007). Natural genetic variation in *Arabidopsis*: Tools, traits and prospects for evolutionary ecology. *Annals of Botany*, 99(6), 1043–1054. <https://doi.org/10.1093/aob/mcl281>
- Subrahmaniam, H. J., Libourel, C., Journet, E. P., Morel, J. B., Muños, S., Niebel, A., Raffael, S., & Roux, F. (2018). The genetics underlying natural variation of plant–plant interactions, a beloved but forgotten member of the family of biotic interactions. *Plant Journal*, 93(4), 747–770. <https://doi.org/10.1111/tpj.13799>
- Swarbrick, P. J., Huang, K., Liu, G., Slate, J., Press, M. C., & Scholes, J. D. (2008). Global patterns of gene expression in rice cultivars undergoing a susceptible or resistant interaction with the parasitic plant *Striga hermonthica*. *New Phytologist*, 179(2), 515–529. <https://doi.org/10.1111/j.1469-8137.2008.02484.x>
- Tillman, D. (1985). The resource-ratio hypothesis of plant succession. *American Naturalist*, 125(6), 827–852. <https://doi.org/10.1086/284382>
- Turkington, R., & Harper, J. L. (1979). The growth, distribution and neighbour relationships of *Trifolium repens* in a permanent pasture: IV. fine-scale biotic differentiation. *The Journal of Ecology*, 67(1), 245. <https://doi.org/10.2307/2259348>
- Umina, P. A., Weeks, A. R., Kearney, M. R., McKechnie, S. W., & Hoffmann, A. A. (2005). Evolution: A rapid shift in a classic clinal pattern in *Drosophila* reflecting climate change. *Science*, 308(5722), 691–693. <https://doi.org/10.1126/science.1109523>
- Weigel, D., & Nordborg, M. (2005). Natural variation in *Arabidopsis*. How do we find the causal genes? *Plant Physiology*. <https://doi.org/10.1104/pp.104.900157>
- Wendling, M., Büchi, L., Amossé, C., Jeangros, B., Walter, A., & Charles, R. (2017). Specific interactions leading to transgressive overyielding in cover crop mixtures. *Agriculture, Ecosystems and Environment*, 241, 88–99. <https://doi.org/10.1016/j.agee.2017.03.003>
- Zhao, K., Aranzana, M. J., Kim, S., Lister, C., Shindo, C., Tang, C., Toomijan, C., Zheng, H., Dean, C., Marjoram, P., & Nordborg, M. (2007). An *Arabidopsis* example of association mapping in structured samples. *PLoS Genetics*, 3(1), 0071–0082. <https://doi.org/10.1371/journal.pgen.0030004>

## *Chapter 1*

# *Establishing a genomic map of local adaptive cooperation in Arabidopsis thaliana*

*“In a world created by natural selection, homogeneity means vulnerability”*

*Edward O. Wilson, 1992*

## I. Introduction

During the course of its life cycle, a plant can interact directly or indirectly – consecutively and/or concurrently - with multiple neighboring plants. Plant–plant interactions play an important role in regulating the diversity and composition of plant communities and ultimately ecosystems functioning through their effects on resource availability and habitat structure (Brooker 2006, Martorell & Freckleton 2014). Studying the mechanisms underlying plant-plant interactions is therefore considered to be essential to understand the dynamics of plant communities, which may in turn help to predict the resilience of plant species in presence of anthropogenic-related global changes (Gilman *et al.* 2010, Singer *et al.* 2013, Subrahmaniam *et al.* 2018).

For decades, interspecific competitive plant-plant interactions have been considered as the major factor for determining the structure of plant communities. However, the role of positive interactions in regulating the assembly of communities and to explainoveryielding in crop mixtures, has gained a lot of attention recently (Bertness & Callaway, 1994, Callaway, 1995, Brooker & Callaghan, 1998, Bruno *et al.* 2003, Kotowska *et al.* 2010, Wendling *et al.* 2017). Overyielding corresponds to an increase in productivity of plants in species mixtures as opposed to species monocultures. Three mechanisms have been put forward to explainoveryielding in species mixtures: sampling effect (Hutson *et al.* 1997), complementarity and facilitation (Hector *et al.* 1999). *Sampling effect* corresponds to the fact that when communities are randomly assembled from a pool of species, diversified mixtures are more likely to contain species with high productivity (Hutson *et al.* 1997). This can lead tooveryielding if these species become dominant in the community. *Complementarity* designates the differentiation of species niches in time and space. Niche differentiation allowed coexistence between species by reducing competitive interactions. Finally, *facilitation* refers to an active interaction between individuals for fulfilling resource requirements and/or stress amelioration.

In wild plant species, whileoveryielding has been well documented at the interspecific level, it has been relatively less highlighted at the intraspecific level. By contrast, underyielding, which corresponds to an increase in the productivity of plants when they grow in monogenotypic culture compared to a mixture of genotypes, a phenomenon directly corresponding to kin

## Chapter 1

cooperation, has been more frequently reported at the intraspecific level (Subrahmaniam *et al.* submitted). Nevertheless, existence of positive interactions including both kin cooperation (KC) and overyielding (OY) has been demonstrated within wild plant species (Subrahmaniam *et al.* submitted). Two main hypotheses, both predicting opposite outcomes for the relationship between the level of genetic relatedness and extent of positive interactions among interacting genotypes have been put forward to explain these two types of positive GxG interactions (Subrahmaniam *et al.* submitted). Kin selection theory predicts partisan help to be given to close relatives. By contrast, according to the elbow-room hypothesis, genetically close relatives will compete for the same resources and increasing genetic distance between genotypes can translate into increasing niche partitioning, thereby leading to overyielding in mixtures of genotypes.

Despite the demonstration of positive interactions (KC and OY) in several wild plant species (Subrahmaniam *et al.* submitted), the underlying genetic architecture is still largely unknown. To our knowledge, only one study has addressed this challenge. By using 37 RILs, a single major effect QTL was detected as underlying kin cooperation in *A. thaliana* (Wuest and Niklaus 2018). While informative, there were two main shortcomings associated with this study. Firstly, the low number of RILs used precluded a proper characterization of the genetic architecture (Keurentjes *et al.* 2007). Secondly, the experiment was based on two parents from worldwide collections of accessions (Bay-0 from The Netherlands and Sha-0 from Tajikistan) to create the experimental RIL population. Positive interactions are supposed to be frequent among genotypes from the same population due to repeated frequent interaction (Nowak 2006). Use of accessions that have not co-existed in nature, creates an ecologically non-realistic setting for testing positive interactions.

In addition, it is still unknown whether polymorphic genes involved in positive interactions at the intraspecific level have been shaped by natural selection. Similarly, the ecological drivers associated with such adaptive positive interactions also remain largely unknown.

## II. Objective

**The first aim of my PhD project was to establish a genomic map of local adaptive cooperation in *Arabidopsis thaliana*.**

To achieve this goal, we set up a field experiment at a site located at the INRA Auzeville campus to study the natural variation of intraspecific GxG interactions among wild whole-genome sequenced populations of *Arabidopsis thaliana* collected from the Midi-Pyrénées region. Following this, based on a GWA mapping approach, we first aimed at fine mapping genomic regions associated with natural variation of positive interactions among populations. Then, by performing a genome scan of spatial genetic differentiation, we tested whether the genomic regions associated with natural variation of positive interactions were presenting signatures of local adaptation. We also intended to correlate the observed variation of positive interactions with the large range of ecological variables characterized on these populations to identify putative selective agents driving positive interactions in these populations. Finally, to start understanding the molecular mechanisms underlying these positive interactions, we discussed the function of candidate genes and compared it with the function of genes associated with cooperation identified in other species.

## III. Materials and methods

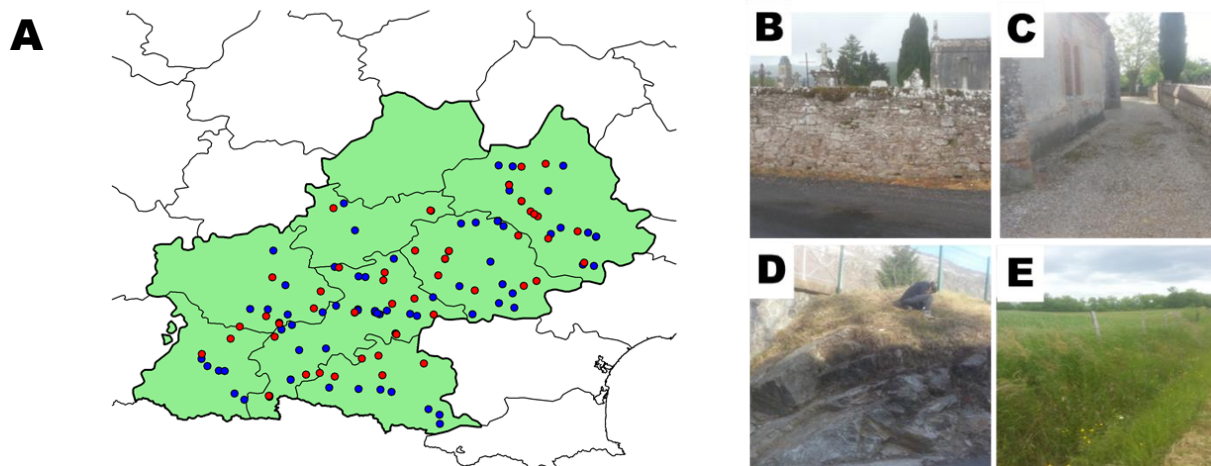
### III.A. Biological material

In this study, we used 54 wild populations of *A. thaliana*, which were among the 168 populations previously identified in the Midi-Pyrénées region by the team (Figure 1; Bartoli *et al.* 2018, Frachon *et al.* 2018). These populations were chosen to maximize the diversity of habitats encountered (meadows, road borders, walls, lawns, bare soil ...) by the species in the Midi-Pyrénées region (Figure 1). Furthermore, these populations have been characterized for a large range of ecological factors such as climate, soil, plant communities and bacterial communities

## Chapter 1

including commensals and pathogens (Bartoli *et al.* 2018, Frachon *et al.* 2018, Frachon *et al.* 2019). For each of the 54 populations, we had 6 climatic variables (Frachon *et al.* 2018), 14 edaphic variables (Frachon *et al.* 2019), 23 *in situ* descriptors of plant communities (diversity, composition and species abundance; Frachon *et al.* 2019) and 44 *in situ* descriptors of bacterial microbiota and bacterial pathobiota (diversity, composition and presence/absence of Operational Taxonomic Units - OTUs; Bartoli *et al.* 2018) (see Annexes 1 and 2 for detailed lists of ecological variables). Of these 44 bacterial descriptors, 27 are associated with bacterial communities in the leaf compartment and 17 with bacterial communities in the root compartment. For plant and bacterial communities, we considered only species or OTUs present in at least 10 of the 54 populations used.

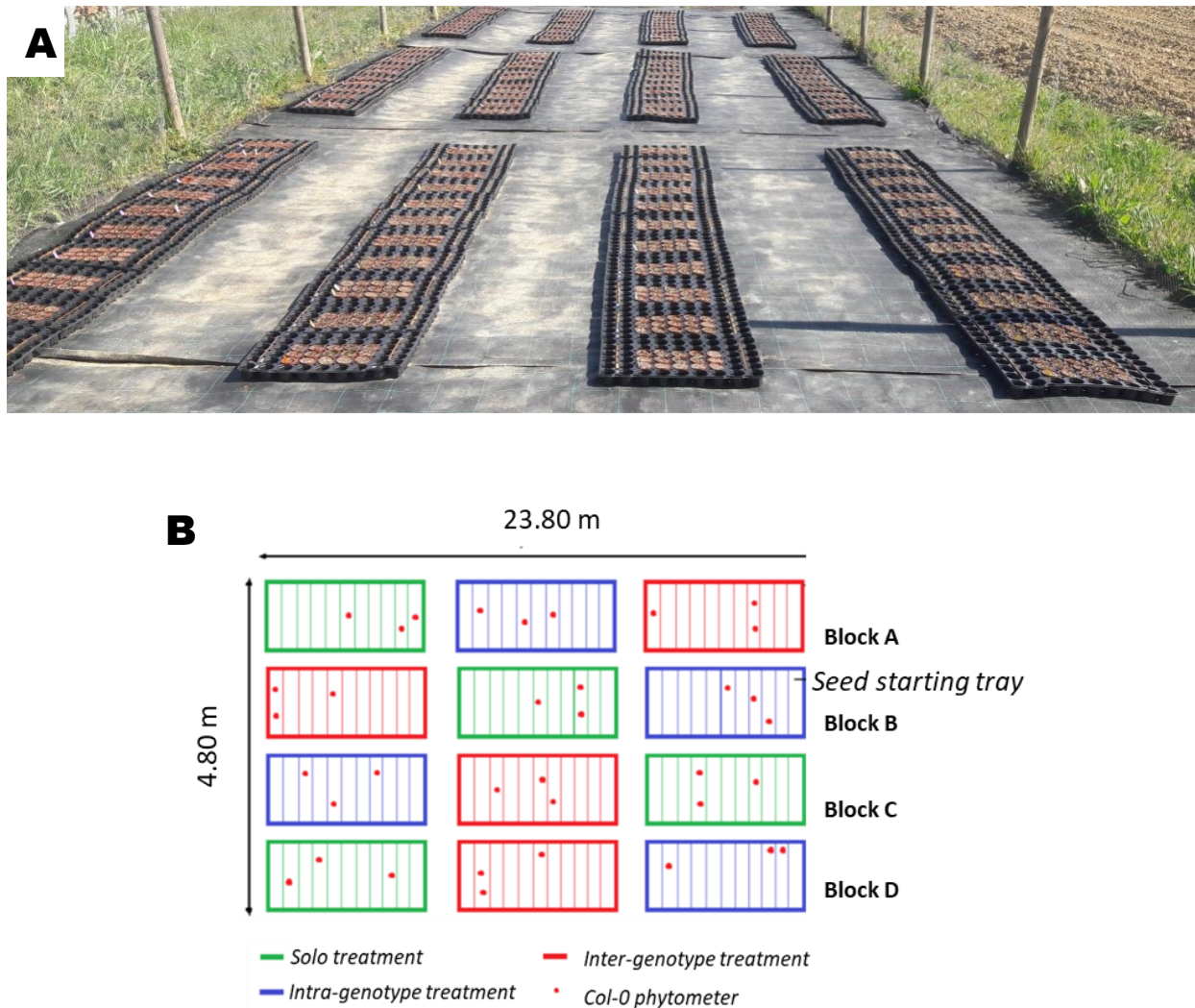
In addition, the choice of working at the regional scale was supported by several advantages presented by the Midi-Pyrénées populations at the genomic level. Firstly, in agreement with previous observations on local populations of *A. thaliana* located in other French regions (Le Corre 2005, Platt *et al.* 2010, Brachi *et al.* 2013), the genome sequencing of the 168 populations by a Pool-Seq approach revealed that all populations were polymorphic (Frachon *et al.* 2018). Secondly, while the main drawbacks in association genetics (*i.e.* effect of population structure on the rate of false positives and false negatives, genetic and allelic heterogeneity, and rare alleles) are often observed at large geographic scales, working at smaller geographic scales should reduce these limitations (Bergelson & Roux 2010).



**Figure 1: Plant material.** A) Map illustrating the position of the 168 natural populations of *A. thaliana* in the Midi-Pyrénées. The 54 populations used for the field experiment are depicted in red. Some habitats of the populations are illustrated with examples as B) wall, C) bare soil, D) lawn and E) meadows.

### III.B. Experimental design

For each of the 54 populations, three random accessions (hereafter named A, B and C) were chosen for this experiment and were grown in three treatments: solo, intra-genotype and intra-population inter-genotype (all pairwise combinations). Differences in the maternal effects among the 162 accessions (54 populations \* 3 accessions) were reduced by growing one plant of each accession for one generation under controlled conditions in 2017 on the INRA Auzeville campus. Each “accession \* treatment” combination was replicated four times in the field according to a Randomized Complete Block Design (RCBD) setup (Figure 2).



**Figure 2: Experimental design.** A) Photograph of the experimental setup. B) Schematic representation of the experimental setup. Phytometers indicated in red dots were arranged randomly.

## Chapter 1

Each "block \* treatment" combination corresponded to the juxtaposition of 11 seed starting trays of 54 cells (9 lines x 6 columns, Figure 2). Within each tray, 15 pots (5 (Ø) x 3 x 4 (H) cm, ~ 51 cm<sup>3</sup>, Soparco®) were placed on the first, third, fifth, seventh and ninth lines and on the first, third and fifth columns (thus leaving a gap among the pots). Each "block \* treatment" combination thus contained 165 pots (11 trays \* 15 pots) that were subjected to randomization.

For the solo treatment, 162 accessions were grown alone (A, B and C). For the intra-genotype treatment, 162 accessions were grown with themselves (AA, BB and CC), with two plants per pot separated by 1cm. For the inter-genotype treatment, we had three intra-population inter-genotype combinations for each of the 54 populations (A-B, A-C and B-C), with two plants per pot separated by 1cm.

For each "block \* treatment" combination, the remaining three pots made it possible to grow the Col-0 worldwide reference accession alone and to use it as a phytometer to control for micro-environmental variations among the experimental plots.

The experiment was conducted from the beginning of March to mid-April 2019 on an experimental site located on the INRA Auzeville campus. The seeds were sown over 4 days (6<sup>th</sup> to 9<sup>th</sup> March 2019) at the rate of one block per day, starting with block A and ending with block D. At least two seeds per position were sown. Each pot was filled with a standard cultivation soil (Soprimex®) with the addition of perlite that retains water in the soil. During the entire growing period, the plants were watered as needed, *i.e.* manual watering morning and evening on hot and dry days and no watering on rainy days. A molluscicide (Ferramol®) was regularly applied around the trays to avoid attacks by slugs and snails. Thinning was carried out within each pot within the first week after seed germination, so that only one plant was present in one given position.

### III.C. Phenotypic trait measurement

The date of germination was daily recorded for each of the accession until the 22<sup>nd</sup> day after sowing. In the 'intra-genotype' and 'intergenotype' treatments, the distance between the two plants in a pot was measured to the nearest millimeter on March 27<sup>th</sup> to verify that it was close to 1 cm (expected distance). With the help of Laurie Tailhades (1<sup>st</sup> year Master student) that I co-supervised with Fabrice Roux, I phenotyped the maximal rosette diameter for each plant 27 days

## Chapter 1

and 35 days after sowing. Rosette diameters were used as a proxy to estimate the aboveground resource accumulation of plants (Baron *et al.* 2015). Rosette diameters were measured twice to estimate the patterns of plant establishment for occupying space in the pot over the course of one week. Thirty-six days after sowing (*i.e.*, when the onset of bolting was observed for the earliest accessions), all accessions were harvested and aboveground dry biomass was estimated for each accession as a proxy for aboveground resource accumulation.

All measurements were expressed according to the phytometers, thus giving relative diameters for the two series of measurements as well as relative biomass for each plant. For each block, the relative values for diameters/biomass were calculated as follows:

$$\text{relative phenotypic value of plant} = \frac{\text{gross phenotypic value}}{\text{average value of phytometers per block}}$$

This conversion into relative estimates made it possible to control for micro-environmental variations between the four blocks.

All analyses were performed on data collected from the ‘intra-genotype’ and ‘inter-genotype’ treatments. The data from the ‘solo’ treatment was collected to check for differences in accession behavior when grown solo *vs* in the presence of another plant (intra or inter-genotype neighbor). The data for the ‘solo’ treatment will be used for answering another question that will not be addressed here.

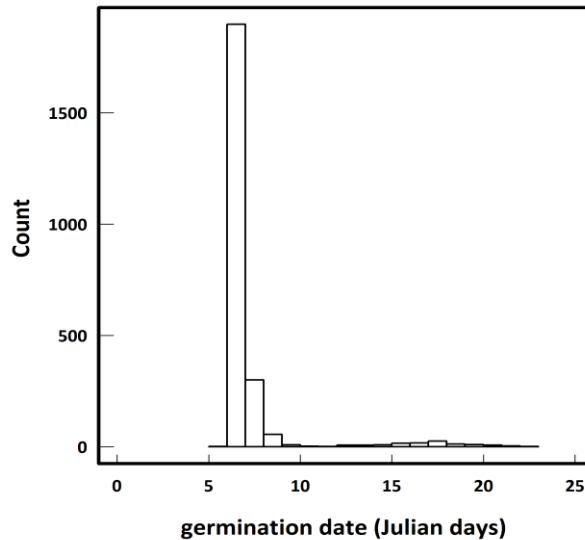
### III.D. Statistical analysis of natural variation of positive interactions

- *Germination date*

A first wave of germination was observed between the 7<sup>th</sup> and the 10<sup>th</sup> day after sowing, with more than 96.7% of germinations occurring during this time, while a second wave of germination spread until the 22<sup>nd</sup> day after sowing (Figure 3). Out of a total of 2,592 individuals planted (54 populations \* 3 accessions \* 2 treatments \* 4 blocks), 264 plants (10.2%) did not germinate. Seeds

## Chapter 1

from two populations (BERNA-A1 and RAYR-B) did not germinate. Therefore, for all further analysis, only data from the 52 remaining populations were considered.



**Figure 3: Distribution of germination date (in Julian days) for all accessions (combining intra- and inter-genotype treatments).**

For all further analysis, we also decided to focus on all the plants that germinated 7 days after sowing, for the following reasons. Firstly, a vast majority of plants germinated on the 7<sup>th</sup> day after sowing (1896 individuals, or 81.4% of the plants). In addition, all the seeds of the Col-0 phytometer germinated on the 7<sup>th</sup> day after sowing. Since all analyses were done based on expressing plant values relative to the average phytometer value within each block, we decided to limit the analyses to all the accessions that germinated on the same day as the phytometer. Secondly, we observed a strong ‘population’ and ‘accession’ effect on germination date (data not shown), explaining about 13.42% of the variance. Therefore, to limit the confounding effects of ‘population’ and ‘accession’ on germination date, we decided to focus only on plants that germinated 7 days after sowing.

- *Intra-genotype treatment*

To explore natural variation among all the accessions from the 52 populations for the relative plant diameters and biomass in the ‘intra-genotype’ treatment, the following generalized linear model was used (PROC MIXED procedure implemented in SAS 9.3, SAS Institute Inc.):

## Chapter 1

$$\text{Model 1: } Y_{ijklmno} = \mu_{\text{trait}} + \text{accession}_i + \text{dist}_j + \text{line}_k * \text{column}_l (\text{block})_m + \text{bolting\_comb}_n + \varepsilon_{ijklmn}$$

In this model, "Y" refers to one of the three phenotypic traits (i.e. first diameter, second diameter and biomass).  $\mu$  is the global phenotypic mean, the term "accession" accounts for differences among all the accessions from the 52 populations. The term "dist" represents the effect of distance between the two plants in a pot and the term "line \* column (block)" represents the effect of the position of the plants within the blocks (knowing that there are 5 rows and 33 columns per block). This latter effect allows taking into account the environmental micro-variations within the blocks. The term "bolting\_comb" was added for the second series of measurements of diameter and biomass. It designates the combined effect of the onset of bolting of the two neighboring plants within a pot on the relative phenotypic mean (at the end of the experiments, about 7.3% of the total surviving plants started to bolt). Finally, the term "ε" is the residual term. All factors were treated as fixed effects.

A significant 'accession' effect was detected for each trait (first diameter:  $F = 1.84$ ,  $P = 1.2 \times 10^{-7}$ ; second diameter:  $F = 1.41$ ,  $P = 0.002$ ; biomass:  $F = 1.57$ ,  $P = 7.62 \times 10^{-5}$ ). The genotypic values of the 156 accessions from the 52 populations were then estimated by calculating least square means (LSmeans) for each trait measured. These genotypic values acquired from the 'intra-genotype' treatment was then used to estimate intergenotypic interactions.

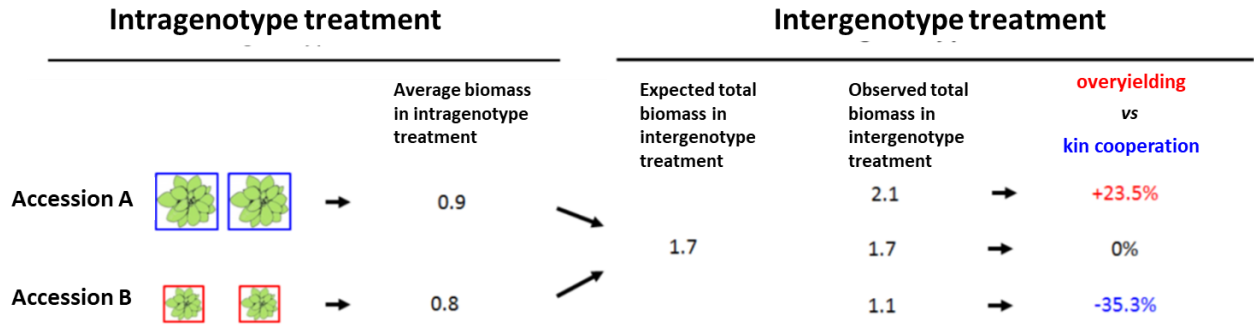
- *Estimating intergenotypic interactions*

For the three phenotypic traits, we estimated inter-genotypic interactions by first summing the relative phenotypic values of both plants per pot in the 'inter-genotype' treatment. Then, we expressed these observed total relative phenotypic values as a function of the expected total relative values estimated from the LSmeans obtained in the 'intra-genotype' treatment.

For each pot in the 'inter-genotype' treatment, the degree of overyielding (OY) / kin cooperation (KC) was therefore calculated as follows:

$$\text{degree of OY/KC} = \frac{(\text{observed intergenotype phenotypic value} - \text{expected intergenotype phenotypic value})}{\text{expected intergenotype phenotypic value}}$$

The obtained values were then converted into percentage values as depicted in Figure 4.



**Figure 4: Illustration of the calculation of the degree of overyielding (OY) / kin cooperation (KC) for each pot of the ‘inter-genotype’ treatment using the hypothetical example of biomass (expressed in mg).**

- *Inter-genotype treatment*

To analyze the natural variation of degree of OY/KC among the 52 natural populations of *A. thaliana*, the following generalized linear model was used (PROC MIXED procedure, SAS):

$$\text{Model 2: } Y_{ijklmno} = \mu_{\text{trait}} + \text{pop}_i + \text{acc\_comb}_j (\text{pop})_i + \text{dist}_k + \text{line}_l * \text{column}_m (\text{block})_n + \text{bolting\_comb}_o + \varepsilon_{ijklmno}$$

where "Y" designates the degree of OY/KC (expressed as percentage values) for one of the three phenotypic traits (i.e. first diameter, second diameter and biomass). The other terms are the same as for Model 1, except for the term "acc\_comb (pop)" which refers to the average effect of the combination of two accessions within populations. All factors were treated as fixed effects. The mean degree of OY/KC per population was obtained by calculating LSmeans.

### III.E. GWA mapping using a Bayesian hierarchical model

To fine map genomic regions underlying OY/KC variation among the 52 populations, we performed a Genome Wide Association mapping using a Bayesian hierarchical model that deals with Pool-Seq data and is implemented in the program BayPass (Gautier 2015). This model takes into account the covariance structure among population allele frequencies that originates from the shared demographic history of populations under study. Following this, we applied a local score

## Chapter 1

approach (Bonhomme *et al.* 2019) to increase the probabilities of detecting minor effect QTLs that could be associated with OY/KC variation.

- *SNP trimming*

Based on a Pool-Seq approach, a representative picture of within-population genetic variation across the genome was previously obtained for the 52 populations used in this study by mapping Illumina reads against the Col-0 reference genome (Frachon *et al.* 2018). The raw data for genome sequencing for each population used in this study have been made available at the NCBI Sequence Read Archive (SRA)<sup>2</sup> through the study accession SRP103198 (Frachon *et al.* 2018). After bioinformatics analysis, the allele read count matrix was composed by 4,781,661 bi-allelic SNPs across the 52 populations.

Following Frachon *et al.* (2018), this matrix was trimmed according to four successive criteria, resulting in a final number of 1,626,275 SNPs. Firstly, SNPs without mapped reads in at least eight populations were removed (number of remaining SNPs = 3,779,668). Secondly, for each population, the relative coverage of each SNP as the ratio of its coverage to the median coverage (computed over all the SNPs in the corresponding population) was calculated. Because multiple gene copies in the 52 populations can map to a unique gene copy in the reference genome Col-0, SNPs with a mean relative coverage across the 52 populations above 1.5 were removed (number of remaining SNPs = 3,241,207). In addition, SNPs with a standard deviation of allele frequency across the 52 populations below 0.004 (number of remaining SNPs = 2,960,221) were also removed. Thirdly, because genomic regions present in Col-0 can be absent in most of the 52 populations or genomic regions present in most of the 52 populations can be absent in Col-0, SNPs with a mean relative coverage across the 52 populations below 0.5 were also eliminated (number of remaining SNPs = 2,885,814). Fourthly, because of bias in GWA/GEA analysis due to rare alleles (Bergelson & Roux, 2010), SNPs that were monomorphic in more than 90% of the populations were also removed, leading to a final data set consisting of read counts for 1,626,275 SNPs in each of the 52 populations.

## Chapter 1

- *BayPass analysis*

Based on the set of 1,626,275 SNPs, whole genome scans for association with OY/KC variation were performed with BayPass 2.1 (Gautier 2015). Dealing with Pool-Seq data, the underlying Bayesian hierarchical model explicitly accounts for the scaled covariance matrix of population allele frequencies ( $\Omega$ ) which make the analyses robust to complex demographic histories (Gautier 2015).

In this study, for each phenotypic trait, we ran the core model to evaluate the association between SNP allele frequencies along the genome of the 52 populations and the observed OY/KC variation (based on LSmeans). For each SNP, we estimated a Bayesian Factor (BF is measured in deciban units) and the associated regression coefficient (Beta\_is,  $\beta_i$ ) using an Importance Sampling algorithm (Gautier 2015). The full posterior distribution of the parameters was obtained based on a Metropolis–Hastings within Gibbs Markov chain Monte Carlo (MCMC) algorithm. A MCMC chain consisted of 15 pilot runs of 500 iterations each. Then, MCMC chains were run for 25,000 iterations after a 2,500-iterations burn-in period. The population LSmeans obtained for the three phenotypic traits were scaled (*scalecov* option) so that  $\mu = 0$  and  $\sigma^2 = 1$ . Because of the use of an Importance Sampling algorithm, we repeated the analyses three times and averaged the values across the three repeats to obtain BF and  $\beta_i$  associated with each SNP.

As previously performed in Frachon *et al.* (2019), we parallelized this genome scan for association with OY/KC variation by dividing the full data set into 32 subdata sets, each containing 3.125% of 1,626,275 SNPs (ca. 50,822 SNPs taken every 32 SNPs along the genome).

- *Estimating genome-wide local score*

This new methodology of estimating genome-wide local score, which is based on the aggregation of GWA mapping results on short physical distances, was recently adapted to plants by our colleague Maxime Bonhomme (LRSV, UPS Toulouse). This local score approach allows detecting significant genomic regions by accumulating the statistical signals from contiguous markers such as SNPs (Fariello *et al.* 2017). In a given QTL region, the association signal, through the  $p$  values, will cumulate locally due to LD between SNPs, which will then increase the local score (Bonhomme *et al.* 2019). Briefly, a sequence of scores is calculated along the chromosome as  $X_i = -\log_{10}(p_i) - \zeta$ , where  $p_i$  is the  $p$  value of marker  $i$  and  $\zeta$  a tuning parameter with an optimal

## Chapter 1

value that can be fixed at 2 or 3 in a GWAS context (Bonhomme *et al.* 2019). Then, finding genomic regions that accumulate strong signals is equivalent to finding peaks along a Lindley process defined as  $h_i = \max(0, h_{i-1} + X_i)$  along the chromosome, with  $h_0 = 0$ . Significant QTL regions and the SNPs they contain (hereafter named top SNPs) are then identified by estimating a chromosome-wide significance threshold for each chromosome (Bonhomme *et al.* 2019). This genome-wide local score approach (GW-LS) allows the detection of QTLs with minor effects (Fariello *et al.* 2017, Bonhomme *et al.* 2019).

In order to apply this methodology on our GWA mapping results, we first ranked each SNP based on the BF values obtained across the genome (from the highest to the lowest values). Then, each rank was divided by the total number of SNPs to obtain a  $p$  value associated with each SNP. The local score approach was then implemented on these  $p$  values to fine map genomic regions associated with OY/KC variation among the 52 populations.

### III.F. Testing for signatures of local adaptation

To test whether the top SNPs identified by GWA mapping combined with a local score analysis present signatures of local adaptation, we first performed a genome-wide selection scan by estimating the XtX measure of spatial genetic differentiation among the 52 populations. For a given SNP, the XtX measures the variance of the standardized population allele frequencies, which results from a rescaling based on the covariance matrix of population allele frequencies (Günther & Coop 2013, Gautier 2015). This allows for a robust identification of highly differentiated SNPs by correcting for the genome-wide effects of confounding demographic evolutionary forces such as genetic drift and gene flow (Gautier 2015). We then tested whether the top SNPs associated with OY/KC variation were over-represented in the extreme upper tail of the XtX distribution according to the methodology described in Brachi *et al.* (2015):

$$FE_{XtX} = \frac{n_a/n}{N_a/N}$$

Here,  $N$  is the total number of SNPs tested genome-wide and  $N_a$  is the total number of top SNPs.  $n$  is the number of SNPs in the upper tail of the XtX distribution. In our case, we used three different thresholds for  $n$ , *i.e.* 0.5% ( $n = 8,132$  SNPs), 0.1% ( $n = 1,626$  SNPs), and 0.01% ( $n = 163$

## Chapter 1

SNPs).  $n_a$  is the number of top SNPs that were also in the upper tail of the XtX distribution. Statistical significance of enrichment was assessed by running 10,000 null circular permutations across the genome based on the methodology described in Hancock *et al.* (2011).

### III.G. Estimating putative ecological drivers of positive interactions

To identify putative ecological drivers of OY/KC variation among populations, we adopted two complementary approaches. With the aim of identifying SNPs that are shared between observed OY/KC variation and ecological variation, we performed a Genome-Environment Association analysis for each of the 87 ecological factors scored on the 52 populations (climate, soil, bacterial and plant communities, Annexes 1 and 2). In a complementary approach, we also performed a sparse Partial Least Square Regression analysis with all the 87 ecological factors in order to identify linear combinations of ecological factors maximizing the covariance with OY/KC variation.

- *Genome Environment Association (GEA)*

The goal of this analysis was to test whether the genomic regions associated with OY/KC variation were also associated with variation of any of the 87 ecological variables, which would in turn help predicting if certain ecological factors could potentially drive adaptive OY/KC among these populations. We adopted the same procedure as described above of combining BayPass analysis with a local score analysis to first identify top SNPs associated with ecological variation among the 52 populations. Following this, we estimated the percentage of top SNPs shared for each ‘ecological factor \* phenotypic trait’ combination. A significant ecology-phenotype association was considered significant when at least 5% of the top SNPs were shared between OY/KC variation and a given ecological factor.

- *sparse Partial Least Square Regression (sPLSR)*

Although GEA is a powerful method for identifying significant associations between genetic polymorphisms and environmental variables, one limitation with this approach is that the analysis is performed on each ecological factor independently. To overcome this issue, we sought to use a complementary approach to help identifying combinations of ecological factors that would

## Chapter 1

maximize the variance of OY/KC variation. Therefore, a sparse Partial Least Square Regression (sPLSR) analysis (a bilinear factor model) was carried out using the mixOmics library implemented in the R environment (Lê Cao *et al.* 2008). This method consists of identifying the combination of ecological variables that, unlike a Principal Component Analysis (PCA), maximize the variance of a dependent variable (Carrascal *et al.* 2009). The dependent variable in this experiment corresponds to the degree of OY/KC and the ecological variables correspond to the 87 variables related to climate, soil, plant communities and bacterial communities. To control for the effect of population structure, we first regressed the LSmeans obtained for OY/KC against the coordinates of the 52 populations on the first PCA genomic axis (explaining 96.4% of the genomic variation observed in the Midi-Pyrénées region) previously obtained in Frachon *et al.* 2018. Following this, sPLSR was carried out on the residuals of OY/KC for detecting significant relationships with combinations of ecological factors.

Based on the methodology developed in Bartoli *et al.* (2018), the significance of the ecological variables included in the linear combinations was estimated from a Jackknife resampling approach by creating 1,000 resampled matrices of 47 populations, i.e. by leaving out 10% of the populations 1,000 times. Only ecological variables with a loading value above 0.2 in more than 75% of the resampled matrices were considered as significant.

## IV. Results

### IV.A. Natural genetic variation of positive GxG interactions

For each phenotypic trait, we detected a significant “population” effect, indicating that our 52 populations differed significantly in the degree of positive interactions (Table 1). The “accession(population)” effect was not significant after a multiple testing correction, suggesting that the three accessions from a given population had on average the same behavior (Table 1). Interestingly, for each phenotypic trait, we observed both OY and KC strategies (Figure 5A). However, the degree of both OY and KC was different between the three traits, with the degree of positives interactions ranging from -31.4% (KC) to +23.5% (OY) for biomass but only from -15.1% (KC) to +15.7% (OY) for the two measurements of diameter (Figure 5A).

## First diameter

Effect	DF num	DF den	F-value	<i>P</i>
<b>Line*column(block)</b>	<b>4</b>	<b>207</b>	<b>21.62</b>	<b>6.40E-15</b>
distance	1	207	5.07	0.025
<b>population</b>	<b>51</b>	<b>207</b>	<b>1.95</b>	<b>5.60E-04</b>
Accession (population)	95	207	1.44	0.015

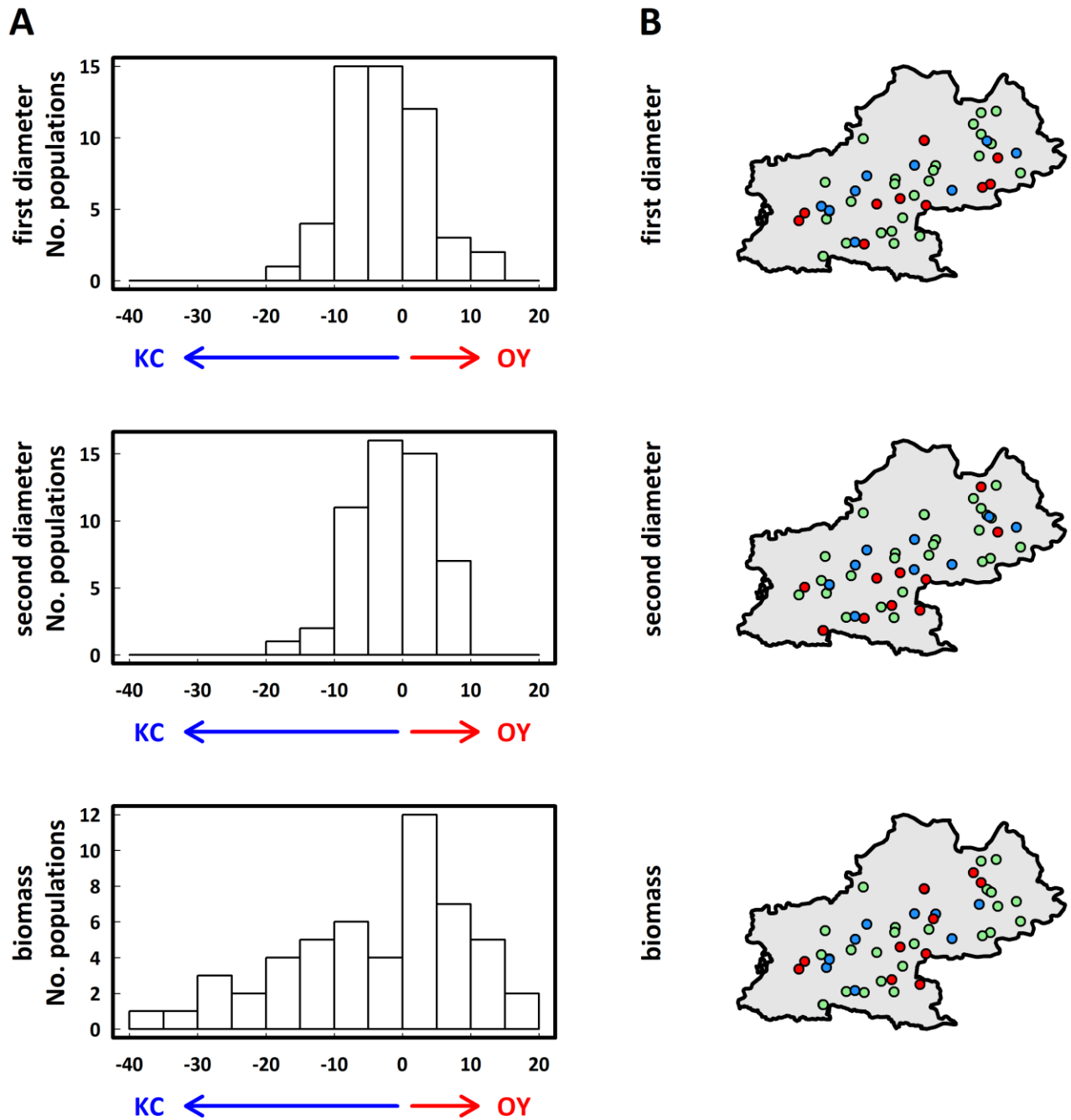
## Second diameter

Effect	DF num	DF den	F-value	<i>P</i>
<b>Line*column(block)</b>	<b>4</b>	<b>195</b>	<b>8.76</b>	<b>1.59E-06</b>
<b>distance</b>	<b>1</b>	<b>195</b>	<b>6.93</b>	<b>0.0091</b>
<b>population</b>	<b>51</b>	<b>195</b>	<b>1.92</b>	<b>7.94E-04</b>
Accession combination (population)	88	195	1.49	0.0116
Bolting combined	14	195	1.95	0.0236

## Biomass

Effect	DF num	DF den	F-value	<i>P</i>
<b>Line*column(block)</b>	<b>4</b>	<b>195</b>	<b>29.07</b>	<b>5.82E-19</b>
distance	1	195	1.37	0.24
<b>population</b>	<b>51</b>	<b>195</b>	<b>1.69</b>	<b>5.64E-3</b>
Accession combination (population)	88	195	1.42	0.021
Bolting combination	14	195	1.81	0.038

**Table 1: Natural variation of intergenotypic interactions based on relative phenotypic trait measurements of rosette diameter at day 27 (first diameter), day 35 (second diameter) and aboveground dry biomass at day 36 (biomass).** The terms 'DF num' and 'DF den' refer to the degrees of freedom of numerator and denominator, respectively. *F*-value: value of ratio of between-group variance to within-group variance. *P*: *p*-value associated with *F*-values. Significant effects after a Bonferroni correction are in bold.



**Figure 5. Natural variation of the degree of overyielding (OY) / kin cooperation (KC) between the 52 natural populations of *A. thaliana*.** A) Histograms illustrating the distribution of the number of populations according to their degree of kin cooperation (<0) vs overyielding (>0) for the three phenotypic traits (first diameter, second diameter and biomass). The degree of OY/KC variation among the 52 populations is expressed in percent. B) Map illustrating the location of the 10 populations showing the highest values for OY (highlighted in light blue) and KC (highlighted in red) for the three phenotypic traits. The other populations are highlighted in green.

The proportion of populations exhibiting either OY or KC also varied between the three phenotypic traits (Figure 5A). For biomass, we observed that the number of populations showing either KC or OY was almost equal (Figure 5A). Between the first and second series of rosette diameter measurements, we detected a shift of ~10% among the populations from the KC strategy to the OY strategy in a span of one week. While 67.3% of the populations showed KC for the first series of measurements, only 57.6% of the populations were found to show this strategy during the second series of rosette diameter measurement (Figure 5A).

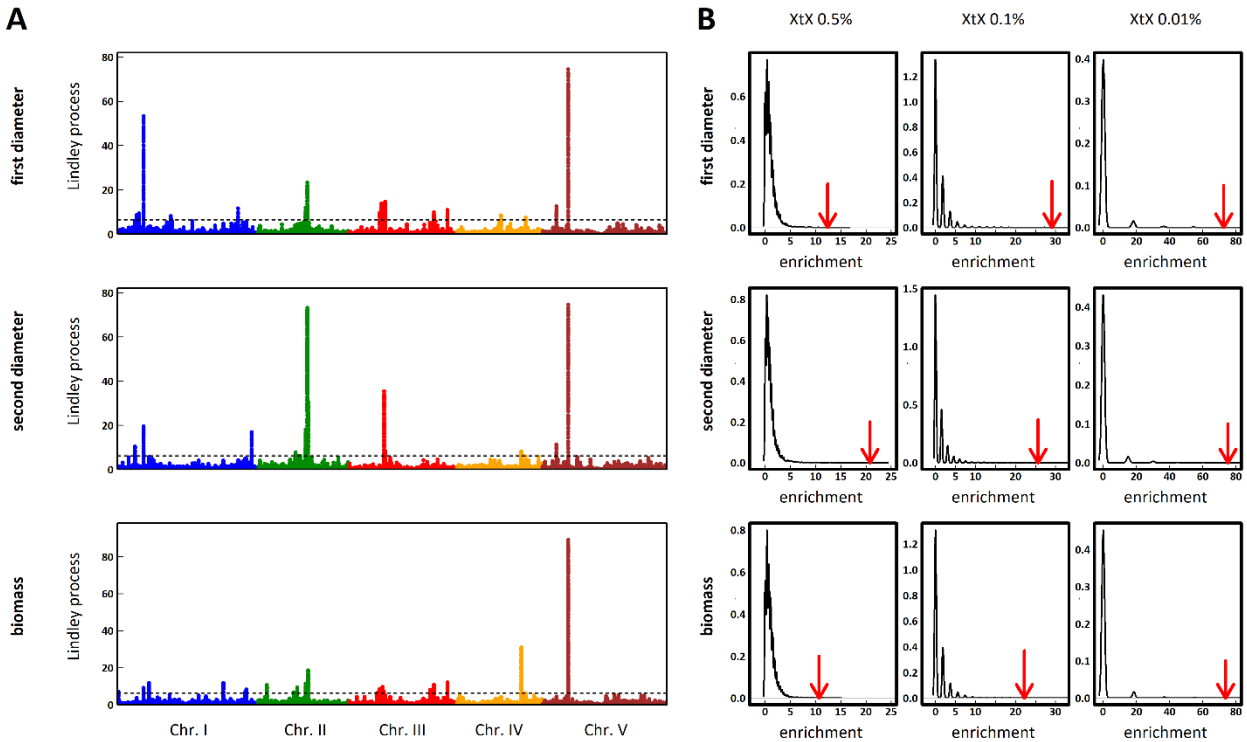
Upon examining the geographic distribution of populations exhibiting either OY or KC strategy in the Midi-Pyrénées region, we observed a fine-grained spatial variation in the degree of OY/KC, with geographically close populations exhibiting opposite type of positive interactions (Figure 5B).

### IV.B. Genetic bases of positive GxG interactions and their adaptive status

By combining a GWA mapping approach based on a Bayesian hierarchical model with a genome-wide local score approach, we identified neat and strong peaks of association for all the three phenotypic traits (Figure 6A, Supplementary Table 1). For the first diameter, we detected 23 QTLs significantly associated with OY/KC variation, each supported on average by ~22 significant SNPs (min = 2, max = 90). The mean QTL size was 1.32kb (min = 3bp, max = 5.15kb). For the second diameter, we detected 21 QTLs, each supported on average by ~30 significant SNPs (min = 4, max = 182). The mean QTL size for OY/KC variation was 1.72kb (min = 18bp, max = 11.64 kb). For biomass, we detected 23 QTLs, each supported on average by ~21 significant SNPs (min = 3, max = 105). The mean QTL size was 1.18kb (min = 3bp, max = 5.77kb).

Across the three phenotypic traits, we identified a total number of 44 QTLs, four of them being shared between all the three traits (Supplementary Table 1). Accordingly, by retrieving all top SNPs associated with OY/KC variation for each phenotypic trait (between 541 and 663 SNPs), 135 top SNPs were found to be shared across the three traits (Figure 7). The four QTLs shared across the three traits were found to be located at the beginning of chromosome 1 (one QTL of

size 2.91kb), in the middle of chromosome 2 (two QTLs of size 2.03kb and 17.28kb) and at the beginning of chromosome 5 (one QTL of size 15.14kb) (Figure 6A).

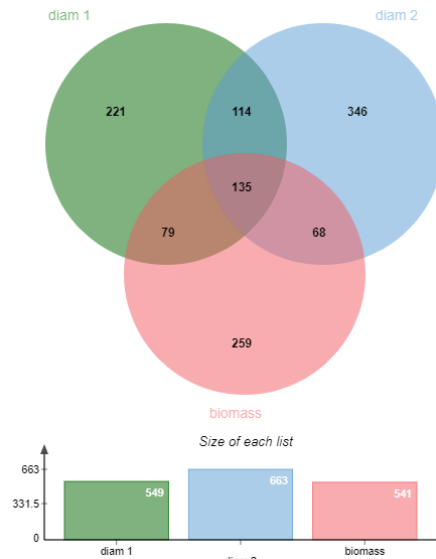


**Figure 6: Genomic maps of local adaptive positive interactions.** A) Manhattan plots of Lindley process values (local score method with  $\xi = 2$ ) for OY/KC variation for first diameter, second diameter and biomass. The x-axis corresponds to the physical position of 1,626,275 SNPs on the five chromosomes. The y-axis designates the value of the Lindley process. The dashed line indicates the significance threshold. B) Density plots illustrating the null distribution (based on 10,000 null circular permutations across the genome) of enrichment values in the upper tail of the XtX distribution according to three thresholds, i.e. 0.5% ( $n = 8,132$  SNPs), 0.1% ( $n = 1,626$  SNPs), and 0.01% ( $n = 163$  SNPs). The position of the red arrows indicate the enrichments values obtained for the top SNPs associated with each phenotypic trait.

To test whether the genomic regions identified by our GWA analysis present signatures of local adaptation, we first performed a genome wide selection scan by estimating XtX measure of genetic differentiation across the 52 populations. We then tested whether the top SNPs associated with OY/KC variation were enriched in the set of SNPs subjected to adaptive spatial differentiation. We detected a significant enrichment of SNPs associated with OY/KC variation in the extreme upper tail of the XtX distribution (Figure 6B). In addition, we observed that the enrichment values increased with the stringency of the cut-off in the upper tail of the XtX distribution, shifting from  $\sim 14$  ( $p$  value  $< 0.001$  across three traits) when considering a threshold of 0.5% to  $\sim 74$  ( $p$  value  $< 0.001$  across three traits) when considering a threshold of 0.01% (Figure

## Chapter 1

6B). Altogether, these results strongly indicate that QTLs associated with OY/KC variation present signatures of local adaptation (Figure 6B).



**Figure 7: Venn diagram showing the number of top SNPs associated with OY/KC variation for each trait (first diameter = diam 1, second diameter = diam 2, aboveground dry biomass = biomass) and the number of SNPs shared between these three traits.**

Out of the 44 QTLs associated with OY/KC variation across the three traits, 11 were found to contain top SNPs that are also present in the 0.5% upper tail of the XtX distribution. Depending on the trait measured (first diameter, second diameter and biomass), out of the 21-23 QTLs identified, between 6 and 7 QTLs are located in genomic regions containing signatures of local adaptation. Three out of the four QTLs shared among the three traits, contained SNPs associated with signatures of local adaptation.

### IV.C. Selective ecological drivers of positive GxG interactions

To identify putative ecological factors driving OY/KC variation among the 52 populations, we adopted two complementary approaches. Firstly, we performed a GEA analysis based on the set of 1,626,275 SNPs using ecological data available for these populations, including 6 climatic variables, 14 edaphic variables, 23 *in situ* descriptors of plant communities (diversity, composition and species abundance) and 45 *in situ* descriptors of bacterial microbiota and pathobiota (diversity,

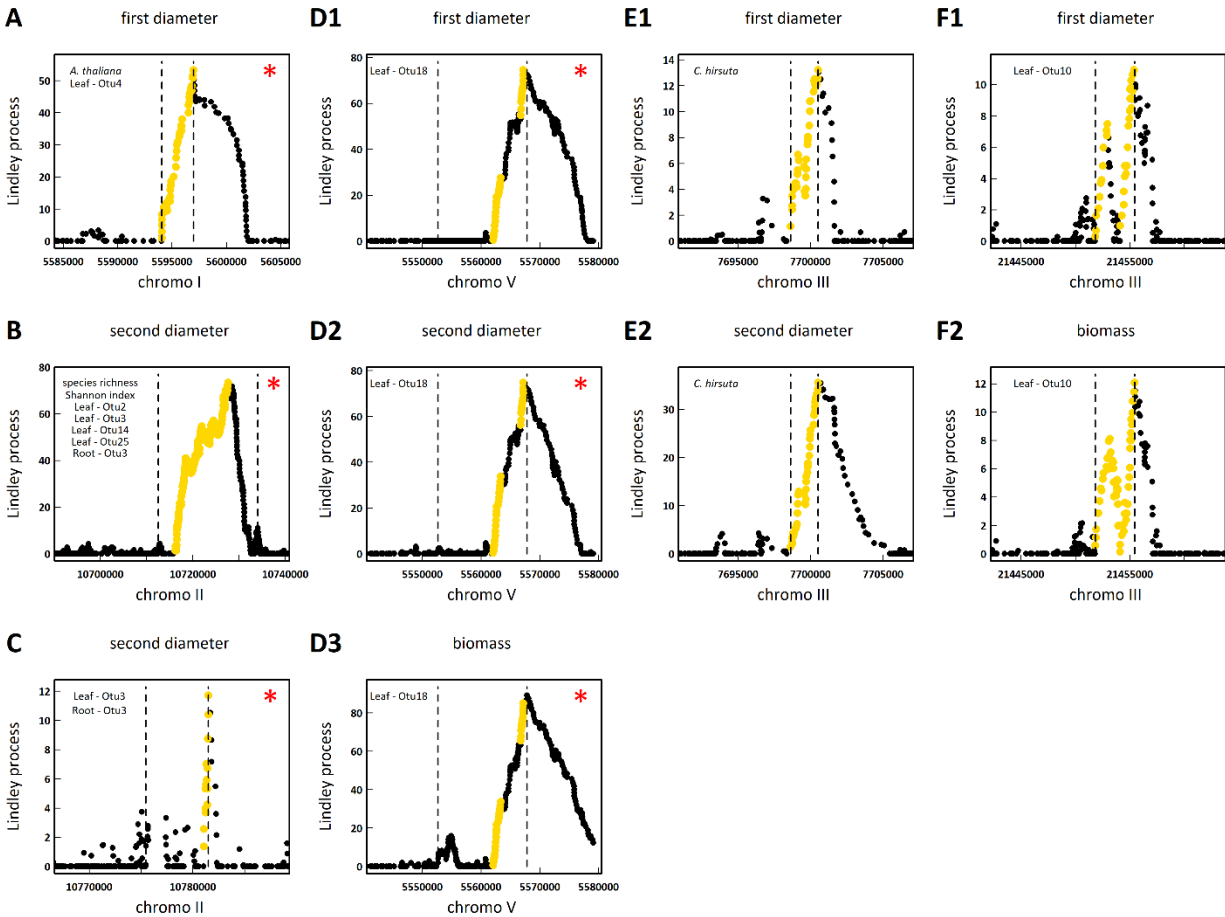
## Chapter 1

composition and OTU presence/absence). Following this analysis, we estimated whether top SNPs associated with natural variation in the degree of OY/KC were also shared with top SNPs associated with any of these ecological factors. We identified 12 ecological variables sharing top SNPs with OY/KC variation, with the number of significant ecological variables varying between 2 (for biomass) and 9 (for second diameter) (Table 2). A non-negligible fraction of top SNPs associated with OY/KC variation can be associated with a specific ecological variable. For instance, 26.5% of top SNPs associated with OY/KC variation for the second diameter were also associated with species richness of plant communities (Table 2).

ecological category	ecological variable	first diameter		second diameter		biomass	
		shared SNPs (%)	PLSR	shared SNPs (%)	PLSR	shared SNPs (%)	PLSR
soil	Manganese concentration	0.0		0.0		0.0	0.35 (9.1%)
	pH	1.8	-0.32 (9.8%)	1.5		0.0	-0.54 (12.3%)
plant communities	species richness	0.0		26.5		0.0	
	Shannon index	1.8		8.3		0.0	0.57 (12.6%)
	abundance of <i>Arabidopsis thaliana</i>	7.3		3.9		3.0	
	abundance of <i>Cardamine hirsuta</i>	6.9		5.7		0.0	
microbial communities	Leaf - <i>Paraburkholderia</i> sp. (Otu2)	0.0		14.2		0.0	
	Leaf - <i>Collimonas</i> sp. (Otu3)	0.7	-0.36 (10%)	18.6	-0.75 (14.7%)	0.7	
	Leaf - <i>Variovorax</i> sp. (Otu4)	6.4	-0.51 (13.2%)	3.8		3.7	
	Leaf - Burkholderiales (Otu10)	6.0		0.0		10.5	
	Leaf - <i>Caulobacter</i> sp. (Otu14)	0.0		6.6		0.0	
	Leaf - <i>Caulobacter</i> sp. (Otu18)	6.4		5.3		6.5	
	Leaf - <i>Sphingomonas</i> sp. (Otu25)	0.0		13.4		0.0	
	Root - <i>Collimonas</i> sp. (Otu3)	0.0		10.1		0.0	
	Root - <i>Pseudomonas fluorescens</i> (Otu6)	0.0	-0.35 (10.1%)	0.0		0.0	

**Table 2: Results obtained for the identification of putative ecological factors driving natural variation of the degree of OY/KC among the 52 populations.** For each phenotypic trait, the left column indicates the percentage of top SNPs associated with OY/KC variation that are also shared with ecological variation. In this study, we only considered as significant the ecological variables sharing more than 5% of the top SNPs with OY/KC variation. These ecological variables are highlighted in light green. For each trait measured, the right column indicates the results of sPLSR with values corresponding to loading values. Only ecological variables with a loading value above 0.2 in more than 75% of the 1,000 Jackknife resampled matrices were considered as significant. The values in brackets indicate the percentage of variance explained by each of the significant ecological variable. The ecological variables with a loading value above 0.2 in more than 90% of the resampled matrices are highlighted in light red.

Interestingly, we observed two opposite outcomes from this analysis. We identified QTLs that are specific to a given phenotypic trait but associated with multiple ecological variables (Figure 8A, B and C), or QTLs associated with several phenotypic traits but associated with the same ecological trait (Figure 8 D, E and F). Surprisingly, all ecological variables identified by this approach correspond to biotic factors (Table 2). In addition, all the significant ecological variables related to microbial communities correspond to the presence of commensal OTUs (i.e. microbiota) but none corresponds to descriptors related to bacterial pathogens (i.e. pathobiota) (Table 2).

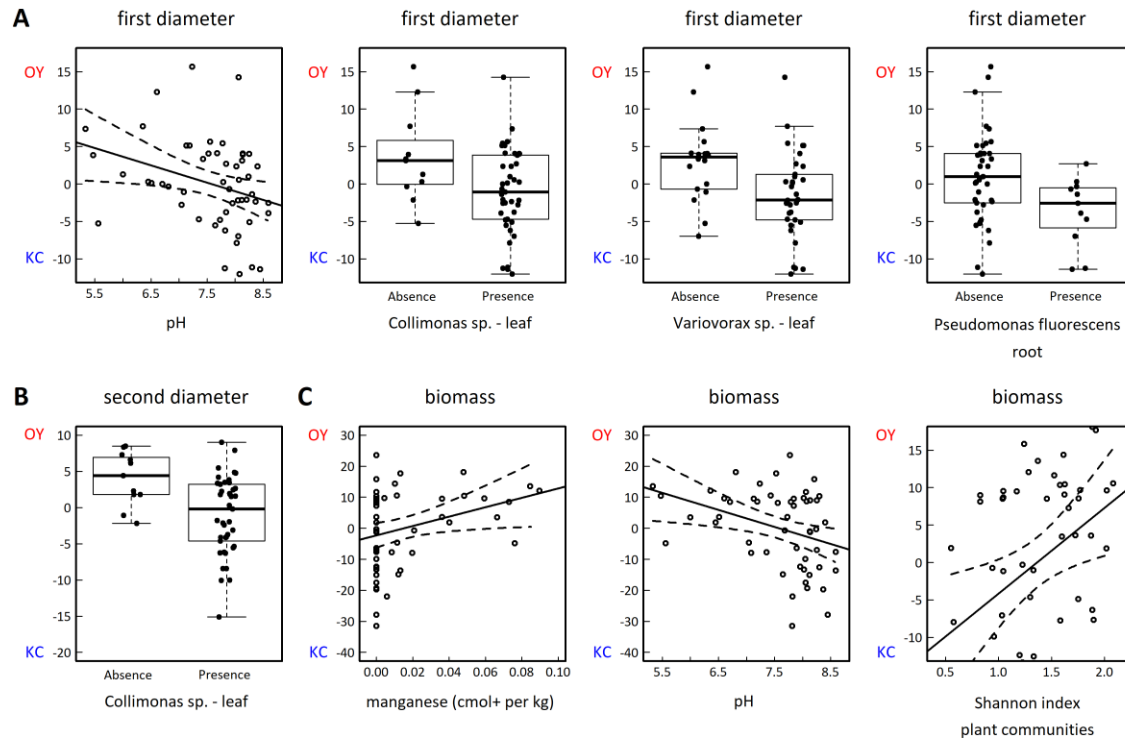


**Figure 8: Identification of putative selective ecological drivers of OY/KC variation among the 52 populations by identification of top SNPs associated with OY/KC variation that are also shared with ecological variation.** A, B and C: Illustration of QTLs associated with OY/KC variation that are also associated with multiple ecological variables. D1, D2 and D3. Illustration of the same QTL region associated with OY/KC variation of the three phenotypic traits and the same ecological variable. E1 & E2 and F1 & F2. Illustration of two QTL regions associated with OY/KC variation of two phenotypic traits and the same ecological variable. The x-axis corresponds to the physical position of the SNPs in the corresponding genomic region. The y-axis designates the value of the Lindley process. The dashed segments indicate the QTL regions. The SNPs highlighted in gold indicate SNPs shared between OY/KC variation and ecological variation. The identity of the ecological factors for which top SNPs are shared is listed on the top left corner. The boxes containing red star correspond to QTLs presenting signatures of local adaptation, i.e. they contain SNPs that are present in the 0.5% extreme upper tail of XtX distribution.

Secondly, in a complementary approach, for identifying linear combinations of abiotic and biotic variables that explain most of OY/KC variation, a sparse Partial Least Square Regression (sPLSR) analysis was conducted on each phenotypic trait to maximize covariance between combinations among the 87 ecological variables and observed OY/KC variation after correction for the effect of population structure. With this approach, we identified six ecological variables significantly associated with OY/KC variation, with the number of ecological variables varying between 1 (for second diameter) and 4 (for first diameter) (Table 2). The significant ecological

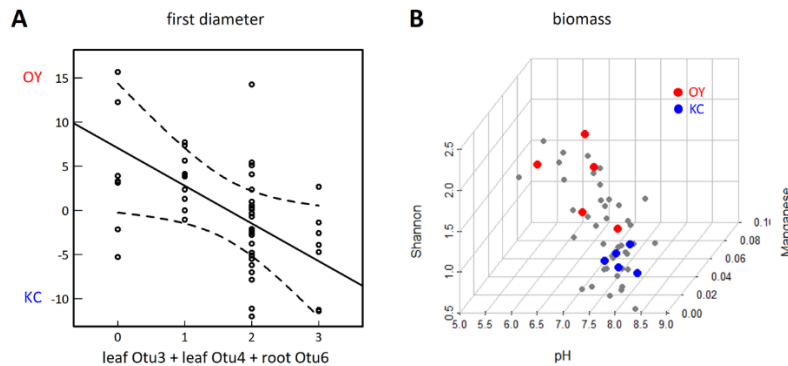
## Chapter 1

variables are related to soil properties ( $n = 2$ ), descriptors of plant communities ( $n = 1$ ) and descriptors of bacterial communities ( $n = 3$ ) (Table 2). Only two ecological factors were linked to more than one phenotypic trait (Table 2). For instance, for first diameter and biomass, the degree of OY/KC was associated with pH variation (Figure 9A and C). For first diameter and second diameter, populations exhibiting the highest values for the KC strategy are found in habitats containing the commensal bacteria *Collimonas sp.* (Figure 9A and B).



**Figure 9: Relationships between OY/KC variation and ecological variation obtained from sPLSR analyses.** A, B and C. Illustration of the significant ecology-phenotype relationships for each of the three phenotypic traits. The degree of OY/KC variation among the 52 populations is expressed in percent.

Interestingly, we observed a cumulative effect of significant ecological factors in explaining OY/KC variation. For instance, 24.4% of the variance in the degree of OY/KC for the first diameter was explained by the cumulative presence of three commensal OTUs (Figure 10A), whereas between 10% and 13.2% of variance was explained by each OTU independently (Table 2). For biomass, populations exhibiting the highest values for the OY strategy are found in habitats presenting concomitantly a low pH, a high concentration in manganese and a high Shannon index of plant communities (Figure 10B).



**Figure 10: Illustration of the cumulative effect of significant ecological factors identified by sPLSR in explaining OY/KC variation.** A) Linear relationship between OY/KC variation among populations for the first diameter and the cumulative presence of three microbial OTUs (leaf – *Collimonas sp.* Otu3 + leaf – *Variovorax sp.* Otu4 + root *Pseudomonas fluorescens* Otu6). The degree of OY/KC variation among the 52 populations is expressed in percent. B) 3D plot illustrating the relationship between OY/KC variation among populations for biomass and the combination of three abiotic (pH and manganese concentration) and biotic (Shannon index of plant communities) factors. The five populations showing the highest values for OY and the five populations showing the highest values for KC are highlighted in red and blue, respectively. The other populations are depicted in gray.

Across the three phenotypic traits, only three ecological factors associated with OY/KC variation were identified by both GEA analysis performed individually on each ecological variable and sPLSR conducted on the whole set of ecological variables (*i.e.* Shannon index of plant communities, presence of the commensal OTU *Collimonas sp.* in the leaf compartment and presence of the commensal OTU *Variovorax sp.* in the leaf compartment; Table 2).

#### IV.D. Genetic bases of local adaptive cooperation in 52 local populations

We classified the 44 QTLs into three categories: (i) category I containing 27 QTLs only identified as associated with one or more phenotypic traits, (ii) category II containing 13 QTLs associated with phenotypic traits and either showing signatures of local adaptation, or associated with ecological factors, and (iii) category III containing four QTLs associated with phenotypic traits, showing signatures of local adaptation and associated with ecological factors (Figure 8A, B, C and D).

To identify candidate genes associated with OY/KC variation, we retrieved 94 annotated genes located within or overlapping with the identified QTLs (Supplementary Table 1). Based on

## Chapter 1

the annotations and the bibliography available, these genes encode for diverse functions: 43 enzymes, 9 transcription factors, 7 transporters, 4 peptides, 2 receptors, and 3 more specific functions (*i.e.* a microRNA, a heat shock protein and an avirulence-induced protein). The remaining genes of our list do not have a clearly defined function.

Interestingly, out of the 94 candidate genes, ~38% are likely to be involved in plant metabolism. For instance, we found a significant amount of genes coding for enzymes that are part of the respiratory chain of mitochondria such as (i) NDB1 (AT4G28220), an NADPH dehydrogenase shown to affect central metabolism and ammonium tolerance (Wallström *et al.* 2014, Podgorska *et al.* 2018), and (ii) GAMMA CA1 (AT1G19580), coding for a subunit of the largest complex of the mitochondrial respiratory chain and whose mutation lead to growth retardation in *A. thaliana* (Soto *et al.* 2015).

We also noticed several genes associated with response to biotic and abiotic stress. For instance, regarding the biotic stress related genes, four genes encode for defensins (AT1G19610, AT2G25185, AT2G25255, AT2G25305), which are small peptides with antimicrobial activities, GGP1 (AT4G30530), a GAMMA-GLUTAMYL PEPTIDASE involved in camalexin and glucosinolate production (Geu-Flores *et al.* 2009), and LOX5 (AT3G22400), a lipoxygenase associated with plant susceptibility to different pests (Nalam *et al.* 2012, Nalam *et al.* 2015). Regarding the abiotic stress related genes, we found for instance two early-responsive to dehydration stress genes (AT3G21620, AT1G69450), a dehydration associated gene (AT3G21600), as well as several other genes implicated in salt tolerance (AtPUB30, AT3G49810 Hwang *et al.* 2015; HAL3A, AT3G18030, Yonamine *et al.* 2004).

In summary, candidate genes associated with QTLs associated with OY/KC variation encode various functions, with a clear preeminence of functions related to the plant metabolism.

## V. Discussion and perspectives

Although GxG positive interactions within wild plant species have been detected in multiple studies (Subrahmaniam *et al.* submitted), we still have little clues about their underlying adaptive genetic architecture. Moreover, the ecological conditions under which intraspecific positive interactions have been selected remain to be fully understood. Here, by combining association genetics, population genomics and ecology, we set up a field experiment based on local populations of *A. thaliana* from the Midi-Pyrénées region to establish a genomic map of local adaptive cooperation and to identify associated putative selective ecological drivers.

### V.A. Natural genetic variation of intraspecific positive interactions

Intraspecific GxG interaction studies designed to test for the presence of positive interactions have corroborated the existence of either OY or KC in a given plant species (Subrahmaniam *et al.* submitted). By contrast, in our study, we observed the existence of both OY and KC strategies within *A. thaliana*. This discrepancy between previous studies and our study may originate from the number of populations used to evaluate GxG interactions, *i.e.* on average 4 populations in the previous studies compared to 52 populations in our study. Interestingly, the coexistence of both OY and KC strategies within the same plant species has also been observed in crops. For example, a recent meta-analysis for analyzing patterns of positive interactions among 386 cultivar mixtures of wheat highlighted the distribution of KC/OY values between -40% (monocultures performing better than mixtures) to +60% (mixtures performing better than monocultures) (Borg *et al.* 2017). The larger range of OY/KC values observed for wheat in comparison with *A. thaliana* in our study may be explained by the use of a larger set of wheat cultivars sampled worldwide.

Our observations of the KC strategy in some local populations of *A. thaliana* can be linked with the hypothesis that selection reduces negative competitive interactions among related individuals in a group (Willson *et al.* 1987, Tonsor 1989, Donohue 2003). Reduced competition among kins with overlapping niches has been demonstrated in multiple studies and have been

## Chapter 1

rationalized based on indirect fitness benefits for kins (inclusive fitness concept) by not investing in costly competitive behaviour (Subrahmaniam *et al.* submitted). On the other hand, our study also revealed in many local populations intraspecific positive interactions among non-kins from the same population. This OY strategy observed during this experiment does not seem to be associated with the hypothesis of sampling effect as only three accessions were used per population, which makes it unlikely that "super productive" accessions were present in many populations. Similarly, the hypothesis of temporal complementarity of niches seems unlikely because all the seeds were sown at the same time. However, accessions occupied the same space in a pot during the course of the experiment and utilized the shared resources available within each pot and yet exhibited positive interactions. One explanation for the observed overyielding in pairwise mixtures can be intraspecific resource partitioning among genotypes based on their physiological capacities and requirements. In such ecological niche partitioning, reduction in the degree of niche overlap reduces competition by maximizing resource use, thereby maximizing fitness among all individuals within the group (i.e., resource use complementarity; Tilman *et al.* 1997). Intraspecific positive interactions exhibited in these populations could therefore correspond to cooperation with reciprocal/ direct benefit (Subrahmaniam *et al.* 2018).

Of particular note, we also observed a shift of ~10% of the populations from the KC strategy to the OY strategy in a span of one week, suggesting that the type of positive interactions may be dynamic during the life cycle in some of these populations. At the interspecific level, plant neighbour interactions have been suggested to be dynamic in nature, switching from competition to facilitation depending on the environmental conditions (Pugnaire and Luque 2001). One can imagine similar transient cooperative links to exist among intraspecific neighbours for stress amelioration (abiotic/ biotic or combination of stresses). In our study, an explanation for the observed shift towards overyielding could therefore be about the assumed depletion of soil resources during plant growth. Plants with the same genotype have similar resource requirements and can compete more strongly to acquire these resources depending on their physiological requirements. This decrease in resources over time could therefore potentially increase the competition between kins, thereby leading to a lower growth rate compared to the growth rate observed in the inter-genotype treatment and therefore to an increased overyielding observed. Consequently, this implies that transient cooperative links with intraspecific neighbours may

evolve for stress amelioration (abiotic/ biotic or combination of stresses) during the life cycle of *A. thaliana* to face fluctuating environments faced by the plants. Future experiments designed to track these environmental fluctuations, like characterizing the amount of soil nutrients consumed by plants over time are still needed to confirm the role of these micro-local factors in driving dynamics of intraspecific positive interactions.

### V.B. Genetic bases of local adaptive positive interactions

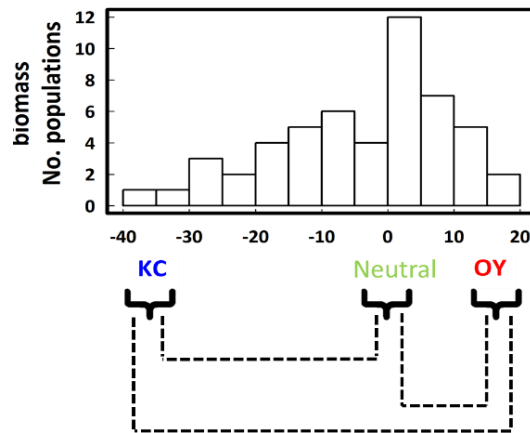
Overall, unlike the monogenic architecture that was described for underlying kin cooperation in an experimental RIL population of *A. thaliana* (Wuest and Niklaus 2018), by using natural populations of *A. thaliana*, our results suggest a polygenic architecture underlying variation of positive interactions among our 52 populations. A possible explanation for this observation could be the low number of RILs used in Wuest & Niklaus (2018), which precluded a proper characterization of the genetic architecture (Keurentjes *et al.* 2007). Moreover, in our study, we implemented a local score approach for increasing the probabilities to detect small QTLs underlying OY/KC variation. The genetic architecture underlying competitive interactions at the interspecific level has previously been described to be polygenic with the detection of medium and small effect QTLs shaping such interactions (Subrahmaniam *et al.* 2018). Our results give insights into a similar genetic architecture underlying variation of positive interactions among local populations.

However, one improvement in the analysis could be made by using a newly statistical test (i.e. contrast test) developed by our colleague Mathieu Gautier (CBGP, Montpellier). This method allows for performing genome scans for genetic differentiation between two groups of populations contrasted either at the phenotypic or at the ecological level (Olazcuaga *et al.* submitted).

The genetic architecture described in our study was obtained by combining all the populations in the same GWA mapping study. However, it is reasonable to assume that the genetic bases underlying OY may differ from the genetic bases underlying KC. Therefore, as depicted in Figure 11, a step in the future direction would be to re-analyze the data by differentiating across

## Chapter 1

the genome extreme OY populations and KC populations and comparing them with populations showing neutral behavior. Pairwise comparisons among OY and neutral, KC and neutral and KC and OY populations could help to better describe the genetic architecture associated with each of the two strategies of cooperation observed among our 52 natural populations.



**Figure 11: Illustration of pairwise genomic comparisons of subsets of populations (populations showing extreme OY values, populations showing extreme KC values, and populations with a neutral behavior) using the contrast test (Olazcuaga *et al.* submitted).**

Importantly, 25% of the detected QTLs carried signatures of local adaptation. In particular, our results indicate the existence of four generalist QTLs that were shared among the three traits, all but one carrying SNPs that were enriched in sets of SNPs subjected to adaptive differentiation. This is the first study, to our knowledge, to demonstrate the existence of local adaptive positive interactions in a wild species. Our regional populations of *A. thaliana* have been previously demonstrated to be adapted to its neighbouring plants at the interspecific level (Frachon *et al.* 2019). The authors reported significant fold enrichment values up to 10.05 for SNPs associated with plant community descriptors (species richness, community composition, presence/absence of the most prevalent plant species) when considering a threshold of 0.1% of the upper tail of spatial differentiation XtX distribution (Frachon *et al.* 2019). Here, based on the same threshold, we observed higher fold enrichment values (between 22 and 29) for the SNPs associated with OY/KC variation across the three traits. This higher fold enrichment for adaptation to intraspecific positive interactions than for interspecific interactions therefore highlights, as previously advocated, the importance of intraspecific GxG interactions in shaping eco-evolutionary patterns of plant communities (Hughes *et al.* 2008, Subrahmaniam *et al.* submitted).

## V.C. Selective ecological drivers of intraspecific positive interactions

Theory suggests that cooperation should exist among individuals of the same population (Nowak 2010) and is expected to be prominent among high stress conditions (Bertness and Callaway 1994). Interspecific facilitation has often been studied with respect to variation of broad scale abiotic factors pertaining to climate or soil (Choler *et al.* 2001, Callaway *et al.* 2002, Maestre and Cortina 2004). By contrast, in agreement with the fine-grained spatial variation in the degree of OY/KC observed in our study, the observed variation of intra-specific positive interactions was mainly linked to biotic factors. This association with ecological factors varying at a small geographic scale may result from the unique set of biotic factors characterized for the 52 populations (Bartoli *et al.* 2018, Frachon *et al.* 2018, Frachon *et al.* 2019). These results arouse the urgent need for a better ecological characterization of natural habitats from which the genetic material is sampled.

In our experiment, we detected a strong and positive relationship between OY/KC variation and Shannon index of plant communities (in native habitats of populations). Similar observations for an OY strategy associated with the presence of native plant communities in the immediate neighbourhood have been made for reed canary grass (*Phalaris arundinacea*), wherein, in a field experiment, genotypes performed better with kins in disturbed plots (where all native vegetation removed) while genotypic mixtures performing better (overyielding) in undisturbed plots (Collins *et al.* 2018). Moreover, in the presence of native vegetation, trait-independent complementarity for occupying interspersed niche spaces was supported in this study. Interestingly, in a meta-analysis conducted by Borg *et al.* (2017), an increase in overyielding observed in wheat mixtures was associated with biotic factors varying at a fine-grained spatial scale (*e.g.*, up to 6.2% in condition of high disease pressures). Altogether, these results indicate that spatial scale of ecological variation to be considered associated with driving intraspecific positive interactions could be similar between crops and wild plant populations.

We also detected a strong and negative relationship between OY/KC variation and the cumulative presence of three OTUs present in either the leaf or the root compartment. Interestingly, it has recently been suggested that in order to understand evolution of cooperative behavior at the

## Chapter 1

intraspecific level, it is important to consider the role of mutualistic microbiota associated with organisms (Kasper *et al.* 2017). This crucial role has been assigned to host associated microbiota as they have direct capacities to alter host behaviors and for their abilities to be transmitted upon social contact (both horizontally and vertically), thus providing positive feedback loop for evolution of sociality (Kasper *et al.* 2017). In addition, in vertebrates, the role of microbiota in influencing social behavior has also been experimentally demonstrated (Klein *et al.* 2003), which is in line with the recent theoretical demonstration describing the putative role of microbes in altruism between organisms (Lewin-Epstein *et al.* 2017).

Our study, although based on a correlational approach, gave strong indications for the dependence of KC on the presence of specific commensal bacteria (Figure 10A). This observation might be in line with previous studies demonstrating recruitment of beneficial microbiota by kins plants grown together for nutrient accumulation from soil (File *et al.* 2012). Plants recruit the soil microbiota by secreting bioactive molecules (root exudates) into the rhizosphere. The composition of root exudates, which is genotype specific, defines the assembly of distinct soil microbial communities. Another hypothesis may rely on the beneficial role of associated microbial communities for defense priming against pathogens (Yuan *et al.* 2018). Thus, this correlation observed for OY/KC variation with the absence/presence of specific microbial OTU is a stepping stone, which deserves to be explored further for dissecting the genuine role of microbiota in shaping evolution of positive interactions within our plant populations. However, we have almost no putative functions associated with the candidate OTUs. Therefore, it is planned to sequence the genome of these OTUs to get access to their gene content, which could help throw light into their putative functions in driving the dynamics of adaptive KC within some of our plant populations.

However, one important limitation in our study comes from the fact that the results obtained with ecological factors are based on correlational approaches. In addition, we need to stress out that the environment experienced by our natural populations cannot be reduced to the only ecological variables described in this study and that co-association with other ecological variables (such as fungi, oomycetes, insects...) might be also considered as an indirect effect of the phenotype-ecology relationships identified in this study. Therefore, in order to validate the causal associations, future experiments have been planned within the team to be conducted in the

same field in spring 2020 by manipulating candidate ecological variables on subsets of populations with extreme behaviors.

### **V.D. Candidate genes underlying the identified QTLs: pre-eminence of functions related to metabolism**

Previous works about natural variation of plant-plant interactions highlighted a substantial proportion of receptors in the list of candidate genes putatively associated with these interactions (Libourel *et al.* biorxiv, Frachon *et al.* 2019). However, we did not find such a proportion in our list of candidate genes as only two genes encode receptors. This might be explained by our specific experimental setup aiming to study cooperation at the intra-population level. Instead, we noticed an extremely high proportion (~38%) of genes involved in metabolism, notably enzymes of the respiratory chain of the mitochondria or related to carbohydrate metabolism, suggesting a major role for these functions in the context of cooperative plant-plant interactions. A possible explanation for this observation would be a complementary use of resources by both partners through their metabolic functions.

We also found a few genes potentially involved the response to biotic and abiotic stress. Interestingly, four defensins, proteins involved in the late stage of defense, were found to be associated with signature of local adaptation. This suggests that some defense functions might be necessary in the context of plant cooperation, potentially for regulatory purposes at the late phases of interaction. On the opposite, the abiotic stress related genes do not present any signature of local adaptation or are not associated with ecological factors. As our field conditions were relatively warm in 2019, we may highlight here genes/QTLs involved in plant-plant cooperation in this particular context.

Among the diverse candidate genes identified in this study, a few of them present functions particularly of interest in the context of cooperative plant-plant interactions. The gene *BE1* (AT3G20440) encodes a putative glycoside hydrolase localized in the chloroplasts, which exerts a possible role in carbohydrate metabolism (Wang *et al.* 2010). This gene controls both embryogenesis and post-embryogenesis growth. *BE1*, through its critical role during the entire plant development cycle might represent a key actor of developmental regulation by controlling

## Chapter 1

homeostasis of some carbohydrates still unknown. In the same line, another metabolic enzyme, a  $\gamma$ -glutamyl peptidase 1 (GGP1, AT4G30530), which increased glucosinolate production by metabolizing a glutathione conjugate (Geu-Flores *et al.* 2009), has been proposed to be part of the camalexin biosynthesis pathway (Moldrup *et al.* 2013). These plant metabolites are well-known for their role in multiple interactions between plants and diverse biotic environments, and might exert a role in plant-plant interactions, especially since their production is influenced by the abiotic environment including the availability of nutrients (in the case of glucosinolates), nitrogen and sulfur (Aarabi *et al.* 2016).

These observations support a view in which plant specialized metabolism can play an essential role in biological processes such as biotic interactions, as classically demonstrated by signal transduction networks. These findings are also in line with our previous detection of a strong and negative relationship between OY/KC variation and the presence of three OTUs in either the leaf or the root compartment. Indeed, plants might recruit and select the microbial members composing their microbiota, *via* bioactive compounds produced through their metabolism (Carvalhais *et al.* 2013).

Another interesting candidate gene is PICKLE (PKL, AT2G25170), which encodes a chromatin remodeling factor (Flaus *et al.* 2006, Ho *et al.* 2013). *PKL* regulates multiple plant development processes, including embryonic development, seed germination, root meristem activity, and hypocotyl cell elongation (Ogas *et al.* 1999, Fukaki *et al.* 2006, Perruc *et al.* 2007, Aichinger *et al.* 2011, Jing *et al.* 2013). Through its function in RNA-directed DNA methylation, this gene might represent a major regulator of development in the context of plant-plant interactions, especially since this is a candidate gene associated with a QTL belonging to category III, *i.e.* associated with phenotypic traits, showing signatures of local adaptation and associated with ecological factors.

These interesting candidate genes must be functionally validated as causal for the corresponding QTLs. This can be achieved by the identification and characterization of mutants (T-DNA, EMS, etc) or by generating transgenic lines with gain or loss of functions for the gene(s) of interest. However, most of the T-DNA mutant lines publicly available have either a Col-0 or a Ws-0 background. Since the experiment discussed here is based on utilizing natural accessions from the Midi-Pyrénées région, an alternative way can be the use of the CRISPR/Cas9 system to

## *Chapter 1*

create gene knockouts. Following this, complementation with natural alleles can then reveal potential Quantitative Trait Polymorphisms involved in driving positive interactions within these local populations. These approaches will help to understand the genetic and molecular mechanisms underlying variation of cooperative interactions between plants.

## VI. References

- Aichinger, E., Villar, C. B. R., Farrona, S., Reyes, J.C., Hennig, L. & Köhler, C. (2009). CHD3 proteins and polycomb group proteins antagonistically determine cell identity in *Arabidopsis*. *PLoS Genetics*, 5: e1000605
- Baron, E., Richirt, J., Villoutreix, R., Amsellem, L. & Roux, F. (2015). The genetics of intra- and interspecific competitive response and effect in a local population of an annual plant species. *Functional Ecology*, 29(10), 1361–1370. <https://doi.org/10.1111/1365-2435.12436>
- Bartoli, C., Frachon, L., Barret, M., Rigal, M., Huard-Chauveau, C., Mayjonade, B., Zanchetta, C., Bouchez, O., Roby, D., Carrère, S. & Roux, F. (2018). *In situ* relationships between microbiota and potential pathobiota in *Arabidopsis thaliana*. *The ISME Journal*, 12: 2024–2038.
- Bergelson, J. & Roux, F. (2010). Towards identifying genes underlying ecologically relevant traits in *Arabidopsis thaliana*. *Nature Reviews Genetics*, 11(12), 867–879. <https://doi.org/10.1038/nrg2896>
- Bertness, M. D. & Callaway, R. (1994). Positive interactions in communities. *Trends in Ecology and Evolution*, 9(5), 187–191. [https://doi.org/10.1016/0169-5347\(94\)90087-6](https://doi.org/10.1016/0169-5347(94)90087-6)
- Bonhomme, M., Fariello, M. I., Navier, H., Hajri, A., Badis, Y., Miteul, H., Samac, D.A., Dumas, B., Baranger, A., Jacquet, C. & Pilet-Nayel, M. L. (2019). A local score approach improves GWAS resolution and detects minor QTL: application to *Medicago truncatula* quantitative disease resistance to multiple *Aphanomyces euteiches* isolates. *Heredity*, 123(4), 517–531. <https://doi.org/10.1038/s41437-019-0235-x>
- Borg, J., Kiær, L. P., Lecarpentier, C., Goldringer, I., Gauffreteau, A., Saint-Jean, S., Barot, S. & Enjalbert, J. (2018). Unfolding the potential of wheat cultivar mixtures: A meta-analysis perspective and identification of knowledge gaps. *Field Crops Research*, 221(September), 298–313. <https://doi.org/10.1016/j.fcr.2017.09.006>
- Brachi, B., Aimé, C., Glorieux, C., Cuguen, J. & Roux, F. (2012). Adaptive value of phenological traits in stressful environments: Predictions based on seed production and laboratory natural selection. *PLoS ONE*, 7(3), e32069. <https://doi.org/10.1371/journal.pone.0032069>
- Brachi, B., Villoutreix, R., Faure, N., Hautekèete, N., Piquot, Y., Pauwels, M., Roby, D., Cuguen, J., Bergelson, J., & Roux, F. (2013) Investigation of the geographical scale of adaptive phenological variation and its underlying genetics in *Arabidopsis thaliana*. *Molecular ecology* 22: 4222-4240
- Brachi, B., Meyer, C. G., Villoutreix, R., Platt, A., Morton, T. C., Roux, F. & Bergelson, J. (2015). Co-selected genes determine adaptive variation in herbivore resistance throughout the native range of *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the United States of America*, 112(13), 4032–4037. <https://doi.org/10.1073/pnas.1421416112>
- Brooker, R. W. (2006). Plant – plant interactions and environmental change. *New Phytologist*, 171(2), 271–284. <https://doi.org/10.1111/j.1469-8137.2006.01752.x>
- Brooker, R.W., & Callaghan, T.V. (1998). The balance between positive and negative plant interactions and its relationship to environmental gradients: A model. *Oikos*, 81, 196–207
- Bruno, J. F., Stachowicz, J. J. & Bertness, M. D. (2003). Inclusion of facilitation into ecological theory. *Trends in Ecology and Evolution*, 18, 119–125
- Bukowski, A. R., Schittko, C. & Petermann, J. S. (2018). The strength of negative plant–soil feedback increases from the intraspecific to the interspecific and the functional group level. *Ecology and Evolution*, 8(4), 2280–2289. <https://doi.org/10.1002/ece3.3755>
- Callaway, R. M. (1995). Positive interactions among plants. *The Botanical Review*, 61(4), 306–349. <https://doi.org/10.1007/BF02912621>

## Chapter 1

- Callaway, R. M., Brooker, R., Choler, P., Kikvidze, Z., Lortie, C. J., Michalet, R., Paolini, L., Pugnaire, F. I., Newingham, B., Aschehoug, E. T., Armas, C., Kikodze, D. & Cook B. J. (2002). Positive interactions among alpine plants increase with stress. *Nature* 417: 844–847.
- Carvalhais, L. C., Dennis, P. G., Fan, B., Fedoseyenko, D., Kierul, K., Becker, A., Von Wiren, N. & Borriss, R. (2013). Linking plant nutritional status to plant-microbe interactions. *PLoS ONE* 8(7), e68555. doi: 10.1371/journal.pone.0068555
- Choler, P., Michalet, R. & Callaway, R. M. (2001). Facilitation and competition on gradients in alpine plant communities. *Ecology* 82: 3295–3308.
- Collins, A. R., Beckage, B. & Molofsky, J. (2018). Small-scale genotypic richness stabilizes plot biomass and increases phenotypic variance in the invasive grass *Phalaris arundinacea*. *Journal of Plant Ecology* 11(1), 47–55. <https://doi.org/10.1093/jpe/rtx056>
- Donohue, K. (2003). The influence of neighbor relatedness on multilevel selection in the Great Lakes sea rocket. *American Naturalist*, 162(1), 77–92. <https://doi.org/10.1086/375299>
- Fariello, M. I., Boitard, S., Mercier, S., Robelin, D., Faraut, T., Arnould, C., Bouchez, O., Salin, G., Dehais, P., Gourichon, D., Leroux, S., Pitel, F., Leterrier, C. & SanCristobal, M. (2017). Accounting for linkage disequilibrium in genome scans for selection without individual genotypes: the local score approach. *Molecular Ecology*, 26: 3700–3714. <https://doi.org/10.1111/mec.14141>
- Flaus, A., Martin, D. M. A., Barton, G. J., & Owen-Hughes, T. (2006). Identification of multiple distinct Snf2 subfamilies with conserved structural motifs. *Nucleic Acids Research*, 34(10), 2887–2905. <https://doi.org/10.1093/nar/gkl295>
- Frachon, L., Bartoli, C., Carrère, S., Bouchez, O., Chaubet, A., Gautier, M., Roby D. & Roux, F. (2018). A Genomic map of climate adaptation in *Arabidopsis thaliana* at a micro-geographic scale. *Frontiers in Plant Science*, 9: 967.
- Frachon, L., Mayjonade, B., Bartoli, C., Hautekeete, N. C. & Roux, F. (2019). Adaptation to plant communities across the genome of *Arabidopsis thaliana*. *Molecular Biology and Evolution*, msz078.
- File, A. L., Klironomos, J., Maherali, H. & Dudley, S. A. (2012). Plant kin recognition enhances abundance of symbiotic microbial partner. *PLoS ONE*, 7(9), 15–17. <https://doi.org/10.1371/journal.pone.0045648>
- File, A. L., Murphy, G. P. & Dudley, S. A. (2012). Fitness consequences of plants growing with siblings: reconciling kin selection, niche partitioning and competitive ability. *Proceedings of the Royal Society B* 279, 209–218.
- Fukaki, H., Taniguchi, N. & Tasaka, M. (2006). PICKLE is required for SOLITARY-ROOT/IAA14-mediated repression of ARF7 and ARF19 activity during Arabidopsis lateral root initiation. *Plant Journal*. 48: 380–389
- Gautier, M. (2015). Genome-wide scan for adaptive divergence and association with population-specific covariates. *Genetics* 201, 1555–1579. doi: 10.1534/genetics.115.181453
- Geu-Flores, F., Nielsen, M. T., Nafisi, M., Møldrup, M. E., Olsen, C. E., Motawia, M. S. & Halkier, B. A. (2009). Glucosinolate engineering identifies a  $\gamma$ -glutamyl peptidase. *Nature Chemical Biology*, 5(8), 575–577. <https://doi.org/10.1038/nchembio.185>
- Gilman, S. E., Urban, M. C., Tewksbury, J., Gilchrist, G. W. & Holt, R. D. (2010). A framework for community interactions under climate change. *Trends in Ecology and Evolution*, 25(6), 325–331. <https://doi.org/10.1016/j.tree.2010.03.002>
- Günther, T. & Coop, G. (2013). Robust identification of local adaptation from allele frequencies. *Genetics* 195, 205–220. doi: 10.1534/genetics.113.152462

## Chapter 1

Hancock, A. M., Brachi, B., Faure, N., Horton, M. W., Jarymowycz, L. B., Sperone, F. G., Toomijan, C., Roux, F. & Bergelson, J. (2011). Adaptation to climate across the *Arabidopsis thaliana* genome. *Science* 334, 83–86. doi: 10.1126/science.1209244

Hector, A., Schmid, B., Beierkuhnlein, C., Caldeira, M., Diemer, M., Dimitrakopoulos, P. G., Finn, J. A., Freitas, H., Giller, P. S., Good, J., Harris, R., Hogberg, P., Huss-Danell, K., Joshi, J., Jumpponen, A., Korner, C., Leadley, P. W., Loreau, M., Minns, A., Mulder, C. P. H., O'Donovan, G., Otway, S. J., Pereira, J. S., Prinz, A., Read, D. J., Scherer-Lorenzen, M., Schulze, E. D., A. S. D., Spehn, E. M., Terry, A. C., Troumbis, A. Y., Woodward, F. I., Yachi, S. & Lawton, J. H. (1999). Plant diversity and productivity experiments in European grasslands. *Science*, 286(5442): 1123–1127

Ho, K. K., Zhang, H., Golden, B. L. & Ogas, J. (2013). PICKLE is a CHD subfamily II ATP-dependent chromatin remodeling factor. *Biochimica et Biophysica Acta*, 1829: 199–210

Hudson, A., Le Corre, V., Loudet, O., Roux, F., Warthmann, N., Weigel, D., Rivero, L., Scholl, R., Nordborg, M., Bergelson, J. & Borevitz, J. O. (2010). The scale of population structure in *Arabidopsis thaliana*. *PLoS Genetics*, 6(2). <https://doi.org/10.1371/journal.pgen.1000843>

Hughes, A. R., Inouye, B. D., Johnson, M. T. J., Underwood, N. & Vellend, M. (2008). Ecological consequences of genetic diversity. *Ecology Letters*, 11(6), 609–623. <https://doi.org/10.1111/j.1461-0248.2008.01179.x>

Hutson M. A. (1997). Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. *Oecologia*, 110(4), 449–460

Hwang, J. H., Seo, D. H., Kang, B. G., Kwak, J. M., & Kim, W. T. (2015). Suppression of *Arabidopsis* AtPUB30 resulted in increased tolerance to salt stress during germination. *Plant Cell Reports*, 34, 277–289.

Jing, Y., & Lin, R. (2013). PICKLE is a repressor in seedling deetiolation pathway. *Plant Signaling and Behaviour* 8: e25026

Kardol, P., Cornips, N. J., Van Kempen, M. M. L., Bakx-Schotman, J. M. T. & Van der Putten, W. H. (2007). Microbe-mediated plant–soil feedbacks causes historical contingency effects in plant community assembly. *Ecological Monographs*, 77, 147–162.

Kasper, C., Vierbuchen, M., Ernst, U., Fischer, S., Radersma, R., Raulo, A., Cunha-Saraiva, F., Wu, M., Mobley, K. & Taborsky, B. (2017). Genetics and developmental biology of cooperation. *Molecular Ecology*, 26(17), 4364–4377. <https://doi.org/10.1111/mec.14208>

Keurentjes, J. J. B., Bentsink, L., Alonso-Blanco, C., Hanhart, C. J., Vries, H. B. De, Effgen, S., Vreugdenhil, D. & Koornneef, M. (2007). Development of a near-isogenic line population of *Arabidopsis thaliana* and comparison of mapping power with a recombinant inbred line population. *Genetics*, 175(2), 891–905. <https://doi.org/10.1534/genetics.106.066423>

Klein, S. L. (2003). Parasite manipulation of the proximate mechanisms that mediate social behavior in vertebrates. *Physiology and Behavior*, 79(3), 441–449. [https://doi.org/10.1016/S0031-9384\(03\)00163-X](https://doi.org/10.1016/S0031-9384(03)00163-X)

Klironomos, J. N. (2002). Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature*, 417, 67–70.

Kotowska, A. M., Cahill, J. F. & Keddie, B. A. (2010). Plant genetic diversity yields increased plant productivity and herbivore performance. *Journal of Ecology*, 98(1), 237–245. <http://doi.org/10.1111/j.1365-2745.2009.01606.x>

Lê Cao K. A., Rossouw, D., Robert-Granié, C. & Besse, P. (2008). Sparse PLS: variable selection when integrating omics data. *Statistical Applications in Genetics and Molecular Biology*, 7, 35.

## Chapter 1

- Le Corre, V. (2005). Variation at two flowering time genes within and among populations of *Arabidopsis thaliana*: comparison with markers and traits. *Molecular Ecology*, 14(13), 4181–4192. <https://doi.org/10.1111/j.1365-294X.2005.02722.x>
- Lewin-Epstein, O., Aharonov, R. & Hadany, L. (2017). Microbes can help explain the evolution of host altruism. *Nature Communications*, 8, 1–7. <https://doi.org/10.1038/ncomms14040>
- Libourel, C., Baron, E., Lenglet, J., Amsellem, L., Roby, D. & Roux, F. (2019). The genomic architecture of competitive response of *Arabidopsis thaliana* is highly flexible between monospecific and plurispecific neighborhoods. *bioRxiv*.
- Maestre, F. T., Callaway, R. M., Valladares, F. & Lortie, C. J. (2009). Refining the stress gradient hypothesis for competition and facilitation in plant communities. *Journal of Ecology*, 97, 199–205.
- Maestre, F. T. & Cortina, J. (2004). Do positive interactions increase with abiotic stress? A test from a semi-arid steppe. *Proceedings of the Royal Society of London, Series B Supplement* 271: S331–S333.
- Martorell, C. & Freckleton, R. P. (2014). Testing the roles of competition, facilitation and stochasticity on community structure in a species-rich assemblage. *Journal of Ecology*, 102(1), 74–85. <https://doi.org/10.1111/1365-2745.12173>
- Møldrup, M. E., Salomonsen, B., Geu-Flores, F., Olsen, C. E., & Halkier, B. A. (2013). De novo genetic engineering of the camalexin biosynthetic pathway. *Journal of Biotechnology*, 167(3), 296–301. <https://doi.org/10.1016/j.jbiotec.2013.06.013>
- Nalam, V. J., Alam, S., Keereetaweep, J., Venables, B., Burdan, D., Lee, H., Trick, H.N., Sarowar, S., Makandar, R., & Shah, J. (2015). Facilitation of *Fusarium graminearum* infection by 9-lipoxygenases in *Arabidopsis* and wheat. *Molecular Plant-Microbe Interactions*, 28(10), 1142–1152. <https://doi.org/10.1094/MPMI-04-15-0096-R>
- Nalam, V. J., Keereetaweep, J., & Shah, J. (2013). The green peach aphid, *Myzus persicae*, acquires a LIPOXYGENASE5-derived oxylipin from *Arabidopsis thaliana*, which promotes colonization of the host plant. *Plant Signaling & Behavior* 8:1 e22735
- Nowak, M. A. (2006). Five rules for the evolution of cooperation. *Science*, 314(5805), 1560–1563. <https://doi.org/10.1126/science.1133755>
- Nowak, M. A., Tarnita, C. E. & Wilson, E. O. (2010). The evolution of eusociality. *Nature*, 466(7310), 1057–1062. <https://doi.org/10.1038/nature09205>
- Ogas, J., Kaufmann, S., Henderson, J. & Somerville, C. (1999). PICKLE is a CHD3 chromatin-remodeling factor that regulates the transition from embryonic to vegetative development in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America*, 96: 13839–13844.
- Olazcuaga, L., Loiseau, A., Parrinello, H., Paris, M., Fraimout, A., Guedot, C., Diepenbrock, L. M., Kenis, M., Zhang, J., Chen, X., Borowieck, N., Facon, B., Vogt, H., Price, D. K., Vogel, H., Prud'homme, B., Estoup, A. & Gautier, M. (2020). A whole-genome scan for association with invasive success in the fruit fly *Drosophila suzukii* using contrasts of allele frequencies corrected for population structure. Submitted.
- Perruc, E., Kinoshita, N. & Lopez-Molina, L. (2007). The role of chromatin-remodeling factor PKL in balancing osmotic stress responses during *Arabidopsis* seed germination. *Plant J.* 52: 927–936.
- Platt, A., Horton, M., Huang, Y. S., Li, Y., Anastasio, A. E., Mulyati, N. W., Agren, J., Bossdorf, O., Byers, D., Donohue, K., Dunning, M., Holub, E. B., Hudson, A., Le Corre, V., Loudet, O., Roux, F., Warthmann, N., Weigel, D., Rivero, L., Scholl, R., Nordborg, M., Bergelson, J. & Borevitz, J. O. (2010). The scale of population structure in *Arabidopsis thaliana*. *PLoS Genetics*, 6(2). <https://doi.org/10.1371/journal.pgen.1000843>

## Chapter 1

Petermann, J. S., Fergus, A. J. F., Turnbull, L. A. & Schmid, B. (2008). Janzen Connell effects are widespread and strong enough to maintain diversity. *Ecology* 89, 2399–2406. doi:10.1890/07-2056.1

Podgórska, A., Ostaszewska-Bugajska, M., Borysiuk, K., Tarnowska, A., Jakubiak, M., Burian, M., Rasmusson, A.G., & Szal, B. (2018). Suppression of external NADPH dehydrogenase—NDB1 in *Arabidopsis thaliana* confers improved tolerance to ammonium toxicity via efficient Glutathione/Redox metabolism. *International Journal of Molecular Sciences*, 19(5), 1–25. <https://doi.org/10.3390/ijms19051412>

Pugnaire, F. I. & Luque, M. T. (2001). Changes in plant interactions along a gradient of environmental stress. *Oikos*, 93(1), 42–49. <http://doi.org/10.1034/j.1600-0706.2001.930104.x>

Reinhart, K. O. & Callaway, R. M. (2006). Soil biota and invasive plants. *New Phytologist*, 170(3), 445–457. <https://doi.org/10.1111/j.1469-8137.2006.01715.x>

Singer, A., Travis, J. M. J. & Johst, K. (2013). Interspecific interactions affect species and community responses to climate shifts. *Oikos*, 122(3), 358–366. <https://doi.org/10.1111/j.1600-0706.2012.20465.x>

Smit, C., Rietkerk, M., & Wassen, M. (2009). Inclusion of biotic stress (consumer pressure) alters predictions from the stress gradient hypothesis. *Journal of Ecology*. 97.1215-1219. <https://doi.org/10.1111/j.1365-2745.2009.01555.x>.

Soto, D., Córdoba, J. P., Villarreal, F., Bartoli, C., Schmitz, J., Maurino, V.G., Braun, H.P., Pagnussat, G.C. & Zabaleta, E. (2015). Functional characterization of mutants affected in the carbonic anhydrase domain of the respiratory complex I in *Arabidopsis thaliana*. *The Plant Journal* 83, 831–844.

Subrahmaniam, H. J., Libourel, C., Journet, E. P., Morel, J. B., Muñoz, S., Niebel, A., Raffaele, S. & Roux, F. (2018). The genetics underlying natural variation of plant–plant interactions, a beloved but forgotten member of the family of biotic interactions. *The Plant Journal*, 93(4): 747-770.

Subrahmaniam, H. J., Roby, D. & Roux, F. (submitted to *Plant Communications*). Towards unifying evolutionary ecology and genomics to understand genotype-by-genotype interactions within wild plant species.

Tilman, D., Lehman, C.L., & Thomson, K.T. (1997). Plant diversity and ecosystem productivity: theoretical considerations. *Proceedings of the National Academy of Sciences of the United States of America* 94:1857-1861.

Tonsor, S. J. (1989). Relatedness and intraspecific competition in *Plantago lanceolata*. *American Naturalist* 134, 897–906. <https://doi.org/10.1086/285020>.

Wagg, C., Boller, B., Schneider, S., Widmer, F. & van der Heijden, M. G. A. (2015). Intraspecific and intergenerational differences in plant-soil feedbacks. *Oikos*, 124(8), 994–1004. <https://doi.org/10.1111/oik.01743>

Wang, X., Xue, L., Sun, J. & Zuo, J. (2010). The *Arabidopsis* BE1 gene, encoding a putative glycoside hydrolase localized in plastids, plays crucial roles during embryogenesis and carbohydrate metabolism. *Journal of Integrative Plant Biology*, 52(3), 273–288. <https://doi.org/10.1111/j.1744-7909.2010.00930.x>

Wallström, S. V., Florez-Sarasa, I., Araújo, W. L., Escobar, M. A., Geisler, D. A., Aidemark, M., Lager, I., Fernie, A.R., Ribas-Carbo, M., & Rasmusson, A. G. (2014). Suppression of NDA-type alternative mitochondrial NAD(P)H dehydrogenases in *Arabidopsis thaliana* modifies growth and metabolism, but not high light stimulation of mitochondrial electron transport. *Plant and Cell Physiology*, 55(5), 881–896. <https://doi.org/10.1093/pcp/pcu021>

Willson, M. F., Hoppes, W. G., Goldman, D. A., Thomas, P. A., Katusic-Malmberg, P. L. & Bothwell, J. L. (1987). Sibling competition in plants: an experimental study. *American Naturalist*, 129, 304–311. <https://doi.org/10.1086/284636>

## Chapter 1

Wendling, M., Büchi, L., Amossé, C., Jeangros, B., Walter, A. & Charles, R. (2017). Specific interactions leading to transgressive overyielding in cover crop mixtures. *Agriculture, Ecosystems and Environment*, 241, 88–99. <https://doi.org/10.1016/j.agee.2017.03.003>

Wuest, S. E. & Niklaus, P. A. (2018). A plant biodiversity effect resolved to a single chromosomal region. *Nature Ecology and Evolution*, 2(12), 1933–1939. <http://doi.org/10.1038/s41559-018-0708-y>

Yonamine, I., Yoshida, K., Kido, K., Nakagawa, A., Nakayama, H., & Shinmyo, A. (2004). Overexpression of NtHAL3 genes confers increased levels of proline biosynthesis and the enhancement of salt tolerance in cultured tobacco cells. *Journal of Experimental Botany*, 55, 387–395

Yuan, J., Zhao, J., Wen, T., Zhao, M., Li, R., Goossens, P., Huang, Q., Bai, Y., Vivanco, J. M., Kowalchuk, G. A., Berendsen, R. L. & Shen, Q. (2018). Root exudates drive the soil-borne legacy of aboveground pathogen infection. *Microbiome*, 6(1), 1–12. <https://doi.org/10.1186/s40168-018-0537-x>

***VII. Supplementary information***

## Chapter 1

**Annex 1:** List of climate, soil, plant community and microbiota/pathobiota associated variables used for sPLSR analyses.

<b>Ecological category</b>	<b>Acronym</b>	<b>Description</b>	<b>Reference</b>
Climate	MAT	Mean annual temperature (°C)	Frachon <i>et al. Front Plant Sci</i> (2018)
Climate	MCMT	Mean coldest month temperature (°C)	Frachon <i>et al. Front Plant Sci</i> (2018)
Climate	PPT_wt	Winter precipitation (mm)	Frachon <i>et al. Front Plant Sci</i> (2018)
Climate	PPT_sp	Spring precipitation (mm)	Frachon <i>et al. Front Plant Sci</i> (2018)
Climate	PPT_sm	Summer precipitation (mm)	Frachon <i>et al. Front Plant Sci</i> (2018)
Climate	PPT_at	Autumn precipitation (mm)	Frachon <i>et al. Front Plant Sci</i> (2018)
Soil	Nitrogen	[Total nitrogen] (g/kg)	Frachon <i>et al. Mol Biol Evol</i> (2019)
Soil	CN	Carbon/nitrogen ratio	Frachon <i>et al. Mol Biol Evol</i> (2019)
Soil	pH	pH	Frachon <i>et al. Mol Biol Evol</i> (2019)
Soil	Phosphorus	[Total Phosphorus] (g/kg)	Frachon <i>et al. Mol Biol Evol</i> (2019)
Soil	Calcium	[Exchange calcium] (cmol+ per kg)	Frachon <i>et al. Mol Biol Evol</i> (2019)
Soil	Magnesium	[Exchange magnesium] (cmol+ per kg)	Frachon <i>et al. Mol Biol Evol</i> (2019)
Soil	Sodium	[Exchange sodium] (cmol+ per kg)	Frachon <i>et al. Mol Biol Evol</i> (2019)
Soil	Potassium	[Exchange potassium] (cmol+ per kg)	Frachon <i>et al. Mol Biol Evol</i> (2019)
Soil	Iron	[Exchange iron] (cmol+ per kg)	Frachon <i>et al. Mol Biol Evol</i> (2019)
Soil	Aluminium	[Exchange aluminium] (cmol+ per kg)	Frachon <i>et al. Mol Biol Evol</i> (2019)
Soil	WHC	Soil water holding capacity (mL/g)	Frachon <i>et al. Mol Biol Evol</i> (2019)
Soil	OC	[Organic carbon] (g/kg)	Frachon <i>et al. Mol Biol Evol</i> (2019)
Soil	SOM	[Soil organic matter] (g/kg)	Frachon <i>et al. Mol Biol Evol</i> (2019)
Soil	Manganese	[Exchange manganese] (cmol+ per kg)	Frachon <i>et al. Mol Biol Evol</i> (2019)
Plant communities	RS_OTU	Species richness	Frachon <i>et al. Mol Biol Evol</i> (2019)
Plant communities	Shannon	Shannon diversity index	Frachon <i>et al. Mol Biol Evol</i> (2019)

## Chapter 1

Plant communities	BLUP_pcoa1_Abund44OTU	Plant community composition – axis 1 PCoA	Frachon <i>et al. Mol Biol Evol</i> (2019)
Plant communities	BLUP_pcoa2_Abund44OTU	Plant community composition – axis 2 PCoA	Frachon <i>et al. Mol Biol Evol</i> (2019)
Plant communities	BLUP_pcoa3_Abund44OTU	Plant community composition – axis 3 PCoA	Frachon <i>et al. Mol Biol Evol</i> (2019)
Plant communities	PC_OTU1	abundance of <i>Conyza canadensis</i>	Frachon <i>et al. Mol Biol Evol</i> (2019)
Plant communities	PC_OTU7	abundance of <i>Taraxacum officinale</i>	Frachon <i>et al. Mol Biol Evol</i> (2019)
Plant communities	PC_OTU20	abundance of <i>Senecio vulgaris</i>	Frachon <i>et al. Mol Biol Evol</i> (2019)
Plant communities	PC_OTU27	abundance of <i>Sonchus oleraceus</i>	Frachon <i>et al. Mol Biol Evol</i> (2019)
Plant communities	PC_OTU46	abundance of <i>Valerianella locusta</i>	Frachon <i>et al. Mol Biol Evol</i> (2019)
Plant communities	PC_OTU65	abundance of <i>Plantago lanceolata</i>	Frachon <i>et al. Mol Biol Evol</i> (2019)
Plant communities	PC_OTU67	abundance of <i>Veronica persica</i>	Frachon <i>et al. Mol Biol Evol</i> (2019)
Plant communities	PC_OTU71	abundance of <i>Veronica arvensis</i>	Frachon <i>et al. Mol Biol Evol</i> (2019)
Plant communities	PC_OTU83	abundance of <i>Convolvulus arvensis</i>	Frachon <i>et al. Mol Biol Evol</i> (2019)
Plant communities	PC_OTU87	abundance of <i>Anagallis arvensis</i>	Frachon <i>et al. Mol Biol Evol</i> (2019)
Plant communities	PC_OTU100	abundance of <i>Sagina apetala</i>	Frachon <i>et al. Mol Biol Evol</i> (2019)
Plant communities	PC_OTU113	abundance of <i>Cerastium glomeratum</i>	Frachon <i>et al. Mol Biol Evol</i> (2019)
Plant communities	PC_OTU132	abundance of <i>Bromus hordeaceus</i>	Frachon <i>et al. Mol Biol Evol</i> (2019)
Plant communities	PC_OTU143	abundance of <i>Festuca rubra</i>	Frachon <i>et al. Mol Biol Evol</i> (2019)
Plant communities	PC_OTU154	abundance of <i>Poa annua</i>	Frachon <i>et al. Mol Biol Evol</i> (2019)
Plant communities	PC_OTU179	abundance of <i>Epilobium sp.</i>	Frachon <i>et al. Mol Biol Evol</i> (2019)
Plant communities	PC_OTU198	abundance of <i>Arabidopsis thaliana</i>	Frachon <i>et al. Mol Biol Evol</i> (2019)
Plant communities	PC_OTU202	abundance of	Frachon <i>et al. Mol Biol Evol</i> (2019)
Leaf microbiota	richness_microbiota_leaf	Species richness	Bartoli <i>et al. ISME</i> (2018)
Leaf microbiota	Shannon_microbiota_leaf	Shannon diversity index	Bartoli <i>et al. ISME</i> (2018)

## Chapter 1

Leaf microbiota	PCOA1_microbiota_leaf	Microbiota composition – axis 1 PCoA	Bartoli <i>et al. ISME</i> (2018)
Leaf microbiota	PCOA2_microbiota_leaf	Microbiota composition – axis 2 PCoA	Bartoli <i>et al. ISME</i> (2018)
Leaf pathobiota	richness_pathobiota_leaf	Species richness	Bartoli <i>et al. ISME</i> (2018)
Leaf pathobiota	Shannon_pathobiota_leaf	Shannon diversity index	Bartoli <i>et al. ISME</i> (2018)
Leaf pathobiota	PCOA1_pathobiota_leaf	Pathobiota composition – axis 1 PCoA	Bartoli <i>et al. ISME</i> (2018)
Leaf pathobiota	PCOA2_pathobiota_leaf	Pathobiota composition – axis 2 PCoA	Bartoli <i>et al. ISME</i> (2018)
Root microbiota	richness_microbiota_root	Species richness	Bartoli <i>et al. ISME</i> (2018)
Root microbiota	Shannon_microbiota_root	Shannon diversity index	Bartoli <i>et al. ISME</i> (2018)
Root microbiota	PCOA1_microbiota_root	Microbiota composition – axis 1 PCoA	Bartoli <i>et al. ISME</i> (2018)
Root microbiota	PCOA2_microbiota_root	Microbiota composition – axis 2 PCoA	Bartoli <i>et al. ISME</i> (2018)
Root pathobiota	richness_pathobiota_root	Species richness	Bartoli <i>et al. ISME</i> (2018)
Root pathobiota	Shannon_pathobiota_root	Shannon diversity index	Bartoli <i>et al. ISME</i> (2018)
Root pathobiota	PCOA2_pathobiota_root	Pathobiota composition – axis 2 PCoA	Bartoli <i>et al. ISME</i> (2018)

**Annex 2:** List of the 29 commensal bacterial OTUs used for sPLSR analyses with the phylum, class, order, family, genus and species taxonomic affiliation (Bartoli *et al.* 2018)

OTU	Phylum	Class	Order	Family	Genus	Species	Reference
SL_Dru00000001	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	<i>Sphingomonas</i>	unclassified	Bartoli <i>et al.</i> /SME/ (2018)
SL_Dru00000002	Proteobacteria	Proteobacteria	Burkholderiales	Burkholderiaceae	<i>Paraburkholderia</i>	unclassified	Bartoli <i>et al.</i> /SME/ (2018)
SL_Dru00000003	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	unclassified	unclassified	Bartoli <i>et al.</i> /SME/ (2018)
SL_Dru00000004	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	<i>Variovorax</i>	unclassified	Bartoli <i>et al.</i> /SME/ (2018)
SL_Dru00000005	Proteobacteria	Proteobacteria	Pseudomonadales	Pseudomonadaceae	<i>Pseudomonas</i>	<i>movaveris</i>	Bartoli <i>et al.</i> /SME/ (2018)
SL_Dru00000006	Proteobacteria	Proteobacteria	unclassified	unclassified	unclassified	unclassified	Bartoli <i>et al.</i> /SME/ (2018)
SL_Dru00000010	Proteobacteria	Proteobacteria	Burkholderiales	unclassified	unclassified	unclassified	Bartoli <i>et al.</i> /SME/ (2018)
SL_Dru00000012	Proteobacteria	unclassified	unclassified	unclassified	unclassified	unclassified	Bartoli <i>et al.</i> /SME/ (2018)
SL_Dru00000013	Proteobacteria	Proteobacteria	Rhizobiales	Methylbacteriaceae	<i>Methylbacterium</i>	unclassified	Bartoli <i>et al.</i> /SME/ (2018)
SL_Dru00000014	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	<i>Caulobacter</i>	unclassified	Bartoli <i>et al.</i> /SME/ (2018)
SL_Dru00000015	Actinobacteria	Actinobacteria	Actinomycetales	Geodermatophilaceae	<i>Geodermatophilus</i>	unclassified	Bartoli <i>et al.</i> /SME/ (2018)
SL_Dru00000018	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	<i>Caulobacter</i>	unclassified	Bartoli <i>et al.</i> /SME/ (2018)
SL_Dru00000025	Proteobacteria	Proteobacteria	Sphingomonadales	Sphingomonadaceae	<i>Sphingomonas</i>	unclassified	Bartoli <i>et al.</i> /SME/ (2018)
SL_Dru00000029	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	<i>Sphingomonas</i>	unclassified	Bartoli <i>et al.</i> /SME/ (2018)
SL_Dru00000035	unclassified	unclassified	unclassified	unclassified	unclassified	unclassified	Bartoli <i>et al.</i> /SME/ (2018)
SL_Dru00000057	Proteobacteria	unclassified	unclassified	unclassified	unclassified	unclassified	Bartoli <i>et al.</i> /SME/ (2018)
SL_Dru00000077	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	unclassified	unclassified	Bartoli <i>et al.</i> /SME/ (2018)
SL_Dru00000093	Proteobacteria	Proteobacteria	Burkholderiales	Comamonadaceae	unclassified	unclassified	Bartoli <i>et al.</i> /SME/ (2018)
SL_Dru00000120	Proteobacteria	Proteobacteria	Rhizobiales	Rhizobiaceae	<i>Rhizobium</i>	unclassified	Bartoli <i>et al.</i> /SME/ (2018)
SR_Dru00000001	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	<i>Sphingomonas</i>	unclassified	Bartoli <i>et al.</i> /SME/ (2018)
SR_Dru00000003	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	<i>Collimonas</i>	unclassified	Bartoli <i>et al.</i> /SME/ (2018)
SR_Dru00000005	Proteobacteria	Proteobacteria	Pseudomonadales	Pseudomonadaceae	<i>Pseudomonas</i>	<i>movaveris</i>	Bartoli <i>et al.</i> /SME/ (2018)
SR_Dru00000006	Proteobacteria	Proteobacteria	Pseudomonadales	Pseudomonadaceae	<i>Pseudomonas</i>	<i>fluorescens</i>	Bartoli <i>et al.</i> /SME/ (2018)
SR_Dru00000007	Proteobacteria	Proteobacteria	Burkholderiales	Oxalobacteraceae	unclassified	unclassified	Bartoli <i>et al.</i> /SME/ (2018)
SR_Dru00000020	Proteobacteria	Proteobacteria	Burkholderiales	Comamonadaceae	unclassified	unclassified	Bartoli <i>et al.</i> /SME/ (2018)
SR_Dru00000049	Proteobacteria	Proteobacteria	Burkholderiales	Oxalobacteraceae	unclassified	unclassified	Bartoli <i>et al.</i> /SME/ (2018)
SR_Dru00000075	Proteobacteria	unclassified	unclassified	unclassified	unclassified	unclassified	Bartoli <i>et al.</i> /SME/ (2018)
SR_Dru00000122	Bacteroidetes	unclassified	unclassified	unclassified	unclassified	unclassified	Bartoli <i>et al.</i> /SME/ (2018)
SR_Dru00000258	Proteobacteria	unclassified	unclassified	unclassified	unclassified	unclassified	Bartoli <i>et al.</i> /SME/ (2018)

**Supplementary Table 1:** Genetic architecture associated with OY/KC variation and relationships with signatures of local adaptation and ecological variables. The list of the 44 QTLs detected across the three phenotypic traits, their physical position and their size are in columns 1-5. QTLs associated with a given phenotypic trait are marked with unique colors for each trait in separate columns 6-8. QTLs presenting signatures of local adaptation are highlighted in column 9. QTLs associated with ecological factors are highlighted in columns 10-21. QTLs have been classified into three categories (column 22) based on (i) association with one or more phenotypic traits (category 1), (ii) association with phenotypic traits and either signatures of local adaptation, or ecological factors (category 2), and (iii) association with phenotypic traits, signatures of local adaptation and ecological factors (category 3 highlighted in blue). The identity of the 94 candidate genes underlying the 44 QTLs is given in columns 23 (ATG number) and 24 (locus name), respectively. The colored cells in column 24 illustrate the different categories of molecular functions (identified locus) of underlying candidate genes, including metabolism (highlighted in green), abiotic (highlighted in yellow) or biotic stress response (highlighted in orange). Cells with a gradation of colors correspond to genes that fall into more than one category of molecular functions. The cells highlighted in purple indicate interesting candidate genes based on their identified functions for validating their putative functioning with respect to OY/KC variation among our 52 populations.

Supplementary Table 1

QTL number	chromosome	start	end	QTL size (Kb)	first diameter	second diameter	biomass	XtX - 0.05%	species richness - plant communities	Shannon index - plant communities	Arabidopsis thaliana	Cardamine hirsuta	Leaf - Parabrachyella sp. (Otu2)	Leaf - Collimonas sp. (Otu3)	Leaf - Variovorax sp. (Otu4)	Leaf - Burkholderiales (Otu10)	Leaf - Caulobacter sp. (Otu14)	Leaf - Caulobacter sp. (Otu18)	Root - Sphingomonas sp. (Otu25)	Root - Collimonas sp. (Otu3)	category	ATG	Locus name
1	1	175036	175181	0,15		x															1	AT1G01471 AT1G01480	ACS2
2	1	3740680	3741471	0,79	x																1	AT1G11160 AT1G11170	KTN80.1
3	1	4078835	4080387	1,55	x																1	AT1G12064 AT1G12070	
4	1	4617786	4619959	2,17	x																1	AT1G13460 AT1G13470	
5	1	5594067	5596976	2,91	x	x	x	x		x				x							3	AT1G16360 AT1G16370	OCT6
6	1	6776079	6783606	7,53		x		x													2	AT1G19580 AT1G19600 AT1G19610 AT1G19620	GAMMA CA1 PDF1.4
7	1	11557354	11558377	1,02	x																1	AT1G32120	
8	1	22924407	22924803	0,40		x															1	AT1G62030	
9	1	26109239	26109623	0,38	x																1	AT1G69450	ERD4
10	1	27629949	27630222	0,27		x															1	AT1G73480	MAGL4
11	1	27898426	27899769	1,34		x															1	AT1G74180 AT1G77350	RLP14
12	1	29071613	29073695	2,08	x							x									2	AT1G77360 AT1G77370	APPR6
13	2	1938654	1938946	0,29		x															1	AT2G05330	
14	2	7768133	7768362	0,23		x															1	AT2G17880	DJC24
15	2	8190326	8190984	0,66		x															1	AT2G18900	
16	2	8535894	8536504	0,61		x	x														1	AT2G19800 AT2G19802 AT2G21390	MIOX2
17	2	9156966	9157254	0,29		x		x													2	AT2G21400	SRS3
18	2	10284380	10284648	0,27	x	x															1	AT2G24190	SDR2
19	2	10372548	10374581	2,03	x	x	x														1	AT2G24370 AT2G24390	
20	2	10561728	10561912	0,18		x															1	AT2G24762 AT2G24765	GDU4 ARF3
21	2	10653318	10653584	0,27		x															1	AT2G25040 AT2G25050	
22	2	10697277	10698669	1,39	x																1	AT2G25130 AT2G25140 AT2G25160 AT2G25169	CLPB4 CYP82F1
23	2	10712543	10734049	21,51	x	x		x	x	x		x	x			x		x	x		3	AT2G25170 AT2G25171 AT2G25180 AT2G25185 AT2G25190	PKL mir862A ARR12

Supplementary Table 1 (continued)

QTL number	chromosome	start	end	QTL size (kb)	first diameter	second diameter	biomass	KtX - 0.05%	species richness - plant communities	Shannon index - plant communities	Arabidopsis thaliana	Cardamine hirsuta	Leaf - Parabrakholderia sp. (Otu2)	Leaf - Collimonas sp. (Otu3)	Leaf - Variovorax sp. (Otu4)	Leaf - Burkholderiales (Otu10)	Leaf - Caulobacter sp. (Otu14)	Leaf - Sphingomonas sp. (Otu18)	Root - Collimonas sp. (Otu25)	category	ATG	Locus name
24	2	10746846	10764121	17,28	x	x	x	x												2	AT2G25220	MYB100
																					AT2G25230	CCP3
																					AT2G25240	
																					AT2G25250	
																					AT2G25255	
																					AT2G25260	HPAT2
																					AT2G25270	
AT2G25280																						
25	2	10775470	10781524	6,05	x	x		x					x							3	AT2G25305	
																					AT2G25310	
																					AT2G25312	
26	2	10860621	10862291	1,67		x						x								2	AT2G25510	
																					AT2G25520	
27	2	10896081	10898402	2,32		x	x													1	AT2G25590	
																					AT2G25600	SPIK
28	3	6168666	6169026	0,36			x	x												2	AT3G18030	HAL3A
																					AT3G18035	HON4
29	3	6715292	6715953	0,66	x		x						x							2	AT3G19380	PUB25
																					AT3G20430	
30	3	7122336	7124539	2,20	x			x												2	AT3G20440	EMB2729
																					AT3G20966	
31	3	7348022	7349196	1,17			x	x												2	AT3G20970	NFU4
																					AT3G21600	
32	3	7610068	7611021	0,95			x													1	AT3G21610	
																					AT3G21620	
																					AT3G21850	SK9
33	3	7698630	7700528	1,90	x	x					xx									2	AT3G21860	SK10
																					AT3G21865	PEX22
																					AT3G22400	LOX5
34	3	7931731	7932232	0,50	x	x														1	AT3G22410	
																					AT3G47810	MAG1
35	3	17642476	17643442	0,97			x													1	AT3G47820	PUB39
																					AT3G49800	
36	3	18472843	18474240	1,40	x		x													1	AT3G49810	ATPUB30
																					AT3G57940	
37	3	21451832	21455456	3,62	x		x						xx							2	AT3G57940	
																					AT4G17050	UGLYAH
38	4	9592865	9592987	0,12	x															1	AT4G17060	FIP2
																					AT4G17090	CT-MBY
39	4	9605995	9606163	0,17	x															1	AT4G28220	NDB1
																					AT4G28230	
40	4	13993297	13996577	3,28		x	x													1	AT4G28230	
																					AT4G28420	
41	4	14056770	14057106	0,34			x	x												2	AT4G28420	
																					AT4G30530	GGP1
42	4	14922737	14924581	1,84	x		x													1	AT4G30540	
																					AT5G09790	ATRX5
43	5	3040031	3040602	0,57	x	x			x											2	AT5G09790	
																					AT5G16890	
44	5	5552676	5567815	15,14	x	x	x	x												3	AT5G16900	
																					AT5G16910	CSLD2
																					AT5G16920	

## Chapter 2

### *Investigating positive GxG interactions at the genomic level in a local population of Arabidopsis thaliana*

*“Every individual matters. Every individual has a role to play. Every individual makes a difference”*

*Jane Goodall, 2017*

## I. Introduction

Both in natural environments and in crop fields, plants rarely grow alone. In fact, during their life cycle, plants can interact simultaneously and/or sequentially, directly or indirectly with multiple plants. Plant-plant interactions are therefore ubiquitous in nature, both at the interspecific level and at the intraspecific level and can be categorized based on the net benefit and cost associated to each interacting partner (Table 1).

Interaction	Nature	Interspecific level	Intraspecific level
Competitive	--	Competition	Competition
Asymmetric	+–	Parasitism Allelopathy	Altruism
Commensal	+0	Facilitation	Cooperation with direct benefit
Reciprocal helping	++	Mutualism (co-adaptation)	Cooperation with reciprocal benefit

**Table 1: Terminology of the different types of interactions among plants defined at the intra and interspecific level (Subrahmaniam *et al.* 2018).**

An interaction is considered competitive when each of the two individuals involved in the interaction receives a cost (-/- interaction), and no benefits (Benefits  $< 0$  and costs  $> 0$ ). There are also asymmetric interactions that concern an individual who obtains a benefit at the expense of another (+/- interaction) (benefit  $< 0$  and cost  $> 0$  for the helper; benefit  $> 0$  and cost  $< 0$  for the receiver). On the other hand, commensal interactions are those where an individual provides help to another (the latter therefore receives a benefit), without being costly for himself (0/+ interaction) (benefit = 0 and cost = 0 for helper; benefit  $> 0$  and cost = 0 for the receiver of the help). Finally, there are also interactions where both partners receive a benefit by interacting (+/+ interaction) (benefit  $> 0$  and cost  $< 0$  for both plant partners). This type of interaction corresponds to reciprocal aid called (i) mutualism when it is an interaction between two species and (ii) cooperation with reciprocal benefits when it is an interaction between two individuals of the same species (Subrahmaniam *et al.* 2018).

In natural plant communities, all these types of interactions can occur simultaneously between different species and even among members of the same species. However, their relative

## Chapter 2

importance in explaining patterns of plant communities is still under debate. For decades, competitive interactions at the interspecific level was recognized as the main type of plant-plant interactions driving the structure of plant communities (Tilman 1985, Goldberg & Barton 1992, Chesson 2000). However, this view has been challenged by multiple recent studies revealing the unsuspected importance of positive plant-plant interactions (Dormann & Brooker, 2002, Bruno *et al.* 2003). In particular, it has been shown that positive plant-plant interactions could also have an important role in the functioning of plant communities and can explain the phenomena of "overyielding", *i.e.* the increase in the productivity of plants when two species grow in mixture compared to a situation monoculture.

Upon decomposing species interactions into interactions occurring between genotypes of species, it has recently been argued that the interaction outcome depends on the genotype identity, rather than species identity (Ehlers *et al.* 2016). Genotype-by-genotype (GxG) interactions at the interspecific level might ultimately govern plant community diversity, composition and structure (Brooker 2006, Ehlers *et al.* 2016). In addition, for a given species, a huge number of genotypes of varying levels of relatedness can co- exist within a local population, even in the case of highly selfing species as it has been recently demonstrated for *A. thaliana* (Platt *et al.* 2010, Brachi *et al.* 2013, Frachon *et al.* 2017). Therefore, the patterns of interactions between different genotypes within one population are bound to vary as well.

In the previous chapter based on a common garden experiment conducted in field conditions, we found that the type and level of intra-population GxG interactions can vary among natural populations of *A. thaliana* sampled at a regional scale. We observed that some populations could benefit from the presence of a kin, thereby giving support for existence of kin cooperation. By contrast, we also observed some populations presenting an overyielding strategy, where an individual could benefit from the presence of a non-kin individual from their own population. Furthermore, this variation in the type and level of intra-population GxG interactions was associated with biotic ecological factors varying at a fine spatial scale. Particularly, overyielding was found associated with higher Shannon index scored in the native populations, which can be imagined as a proxy for interspecific competition. This then begs the question, if supremely competitive environments could be associated with having certain genotypes that can form positive links within the population, and more so, depend on such links for deriving mutual benefits,

## Chapter 2

thereby being “*super overyielders*” in pairwise intergenotypic interactions. These genotypes are called so with the expectation that their performance in the presence of a neighbor could be even better than when they are allowed to grow alone, indicating that positive GxG links with a neighboring genotype are essential for its own increased productivity. Moreover, if such “super overyielding” combinations do occur within a local population, what could be the genetic relationships underlying such couples?

To explain reciprocal altruism among social organisms at the genetic level, mainly three hypotheses can be proposed. Firstly, for the ‘*kin selection*’ hypothesis, if cooperative pairs are relatives sharing a huge chunk of their genome, it could be advantageous for them to cooperate to indirectly increase their inclusive fitness, by the representation of their genes in the next generation. Secondly, for the ‘*greenbeard effect*’ hypothesis, if the cooperative pairs share a gene / set of closely linked genes (greenbeard genes) for social behavior and that they are somehow able to recognize and discriminate between the carrier and non carrier accessions of this ‘greenbeard gene(s)’, then it could ensure a nepotistic behavior in favor of other carrier accessions. Thirdly, the ‘*compatibility genes*’ hypothesis is derived from the observation of metabolite cross-feeding in microbial interactions, where synergistic interactions that involve unrelated individuals or different species reciprocally exchange metabolites such as sugars, growth factors, or amino acids with each other (D’Souza *et al.* 2018). In the previous chapter, we discussed overyielding in term of niche partitioning based on resource use complementarity. Moreover, a significant portion of metabolism related candidate genes was identified for underlying positive interactions in some populations.

One can postulate, based on observations from the prokaryotic world, a similar hypothesis where costly metabolic resources could be shared among genotypes. Deriving complementary benefits through share of metabolites reduces individual costs associated with producing specialized metabolites for each partner, and can thus limit intraspecific conflict among genotypes. Consequently, the ‘super overyielding’ strategy can too prevail in such a scenario.

## II. Objective

**The main goals of this second experimental chapter was to identify the presence of a “super overyielding” strategy in *A. thaliana* and to characterize its underlying genetic architecture.**

To do so, a greenhouse experiment was set up to estimate the level of natural variation of intraspecific interactions within a local population located in a highly diverse and competitive environment. We focused on the highly genetically polymorphic French local population TOU-A, from which 195 whole-genome sequenced accessions have been sampled in 2002 and 2010 (Frachon *et al.* 2017). These 195 accessions were grown either alone or in presence of each of three other partner accessions (hereafter named tester accessions) from the same population. Following this, a GWA mapping approach combined with a local score analysis was adopted to detect QTLs associated with the “super overyielding” strategy. Based on these QTLs, we were able to test three complementary hypotheses for explaining the genetic relationships underlying reciprocal helping between some accessions in this local population, *i.e.* (i) ‘kin selection’ hypothesis, (ii) ‘greenbeard effect’ hypothesis, and (iii) ‘compatibility genes’ hypothesis.

NB: this chapter is based on a greenhouse experiment that was conducted by Cyril Libourel (a former PhD student in the team) and Arnaud Chevalier-Mairet (his M1 internship student). In this chapter, I participated to the statistical analysis on the natural genetic variation of GxG interactions, I run the GWA mapping analysis combined with a local score analysis and I tested the three hypotheses mentioned above.

## III. Materials and methods

### III.A. Biological material

To study local natural genetic variation of intraspecific GxG interactions in *A. thaliana*, a set of 195 accessions collected from a local population located in Toulon-sur Arroux in Burgundy (GPS coordinates: 46 ° 38'53.80 "N - 4 ° 7'22.65" E) were used (Supplementary Table 1). This

## Chapter 2

population named TOU-A has been previously characterized within the team to be highly polymorphic at the phenotypic and genomic level (Platt *et al.* 2010, Huard-Chauveau *et al.* 2013, Debieu *et al.* 2016, Baron *et al.* 2015, Frachon *et al.* 2017). The population is located in a highly diverse (> 20 plant species) and competitive environment (Figure 1, personal observation from Fabrice Roux). Based on our previous results at the Midi-Pyrénées regional scale, where overyielding was positively linked to plant community diversity, the TOU-A population thus appears as an appropriate choice to investigate the presence and extent of intraspecific positive interactions.

Among the 195 accessions, 80 were collected in 2002 and 115 in 2010, respectively named TOU-A1 and TOU-A6 (Supplementary Table 1). The maternal effects of the 195 accessions were reduced by the growth of each accession during one generation, under controlled conditions in a greenhouse (photoperiod of 16h, 20°C) at the beginning of 2011 at the University of Lille 1. Given the outcrossing rate of ~6% observed in the TOU-A population (Platt *et al.* 2010), the 195 accessions were considered as relatively homozygous across the genome. Genome sequencing of the 195 accessions was performed on an Illumina HiSeq2500 using a paired-end read length of 2x100pb with the Illumina TruSeq SBS v3 Reagent Kits (Frachon *et al.* 2017). After several bioinformatics analyses, a matrix of 1,902,592 SNPs was obtained for all 195 accessions (Frachon *et al.* 2017).



**Figure 1: Location of the local population TOU-A located in the village of Toulon-sur-Arroux (Burgundy).** The set of 195 accessions were collected under a 300m electric fence separating two permanent meadows.

## Chapter 2

A Principal Component Analysis (PCA) was run on this matrix using SNPreLate package implemented in the *R* environment. The two first PCA axes revealed a genomic space with a triangular shape (Supplementary Figure 1). In order to study GxG interactions within the TOU-A population, we chose three accessions (TOU-A1-137, TOU-A6-18, TOU-A6-55), each located in one of the three corners of the genomic space (Supplementary Figure 1). These three accessions will be referred to as ‘tester’ accessions from now on.

### III.B. Experimental design

The experiment took place from the end of January to mid-February 2016 in greenhouse conditions (22°C under natural light supplemented by artificial light to provide a 14-hour photoperiod) at the phenotyping platform Toulouse Plant Microbe Phenotyping Platform (TPMP) located on the INRA research center in Auzeville.

The experiment was set up according to a split plot design carried out with three blocks and the following four treatments randomly distributed within each of the three blocks (Figure 2):

- ‘solo’ treatment’ where each of the 195 accessions were grown individually
- ‘intergenotype A1-137’ treatment where each of the 195 focal accessions were grown with the tester TOU-A1-137
- ‘intergenotype A6-18’ treatment where each of the 195 focal accessions were grown with the tester TOU-A6-18
- ‘intergenotype A6-55’ treatment where each of the 195 focal accessions were grown with the tester TOU-A6-55

In total, 4176 seeds sown were distributed in a total of 2394 pots, arranged on a total of 48 trays (*i.e.*, 4 trays per sub-block). For each sub-block, we had for the:

- ‘solo’ treatment (a single plant per pot): 4 pots for each tester accession and 1 pot for the remaining 192 accessions
- ‘intergenotype A1-137’ treatment (two plants per pot): 4 pots for the tester accession TOU-A1-137 and 1 pot for the remaining 194 accessions.

## Chapter 2

- ‘intergenotype A6-18’ treatment (two plants per pot): 4 pots for the tester accession TOU-A6-18 and 1 pot for the remaining 194 accessions.
- ‘intergenotype A6-55’ treatment (two plants per pot): 4 pots for the tester accession TOU-A6-55 and 1 pot for the remaining 194 accessions.

All plants were sown in Ø5cm \* H6cm pots filled with standard potting soil (PROVEEN MOTTE 20, Soprimex®). The soil was treated once with larvicide (VectoBac®). Two seeds were planted 1 cm apart for the three intergenotypic treatments, whereas a single seed was put in the center of the pot for the ‘solo’ treatment. Furthermore, seeds of the 195 accessions were sown in additional pots in order to transplant seedlings that did not germinate. Watering the pots, without addition of external fertilizers, was done every two days by filling the trays with water.

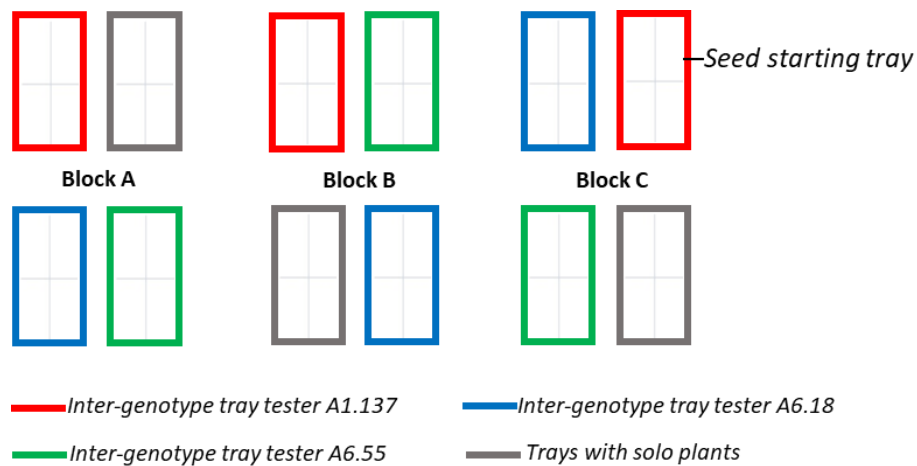


Figure 2: Schematic representation of the split plot design used in this study.

### III.C. Phenotypic trait measurement

The date of germination was noted for each of the seeds sown. Six days after sowing, the seeds that did not germinate were transplanted with seedlings from the additional pots. This transplanting was repeated a few days later for the few transplants that died. Some accessions did not germinate at all (either in the experiment pots or in the additional pots) and were therefore removed from the experiment (n = 17).

As a proxy for aboveground resource accumulation, the largest diameter of the rosette was measured on each plant. The measurements were taken on all the plants of the three ‘intergenotype’ treatments 21 days after sowing (*i.e.*, when the onset of bolting was observed for the earliest accessions), and on all the plants of the ‘solo’ treatment 22 days after sowing. In order to ensure that there were no impact on further analyses due to the two measurement dates, some of the plants measured on the first day were also measured on the second day and similar values were obtained (data not shown). In the three ‘intergenotype’ treatments, rosette diameter was measured both on the tester plants and on the focal plants in order to estimate both direct (effect of testers on focal plants) and indirect (effect of focal plants on testers) response associated with intraspecific interactions.

### III.D. Statistical analysis of natural variation of positive interactions

- *Effect of transplantation*

Significant statistical effects due to transplantation were detected (data not shown). The pots with transplanted seedlings were therefore excluded from the analyzes, *i.e.* 19.3%, 32.6%, 31.1%, and 27.8% of the pots containing the 'solo', ‘intergenotype A1-137’, ‘intergenotype A6-18’ and ‘intergenotype A6-55’ treatments, respectively. We therefore obtained the measurements of rosette diameter for 178, 168, 169, and 171 accessions for the ‘solo’, ‘intergenotype A1-137’, ‘intergenotype A6-18’ and ‘intergenotype A6-55’ treatments, respectively.

- *Natural variation of GxG interactions*

To study the natural genetic variation of the response of focal accessions to the presence of the three tester accessions (*i.e.* direct effects), the following model (PROC MIXED procedure, REML method, SAS 9.3, SAS Institute Inc.) was used:

$$\text{Model 1: } Y_{ijklmn} = \mu_{\text{trait}} + \text{block}_i + \text{treatment}_j + \text{block}_i \times \text{treatment}_j + \text{generation}_k + \text{accession}_l (\text{generation}_k) + \text{treatment}_j \times \text{generation}_k + \text{treatment}_j \times \text{accession}_l (\text{generation}_k) + \text{germ}_m + \text{diff}_n + \epsilon_{ijklmn}$$

## Chapter 2

Here, "Y" is the diameter measured on the focal plants, " $\mu$ " is the global phenotypic mean; "block" represents the micro-environmental differences between the three experimental blocks; "treatment" corresponds to the effect of the four treatments (solo, A1-137, A6-18, A6-55); "generation" refers to the differences between TOU-A1 accessions collected in 2002 and TOU-A6 accessions collected in 2010; "accession (generation)" represents the genetic variation between accessions within each generation; "germ" refers to the date of seed germination of the focal plants; "diff" represents the difference for the date of seed germination between the focal plants and the tester plants; and  $\epsilon$  is the residual term.

The 'diff' effect in *Model 1* cannot be tested for the "solo" treatment because it only contains one plant per pot. Moreover, upon only considering the three intergenotype treatments, the 'diff' effect was not significant (data not shown). We therefore decided not to include it in the full model based on the four treatments. All effects were considered fixed. For the calculation of *F*-values, terms were tested over their appropriate denominators. Given the split-plot design used in this study, the variance associated with 'block x treatment' was used as the error term for testing the 'block' and 'treatment' effects.

To study the natural genetic variation of the response of the three tester accessions to the presence of focal accessions (*i.e.* indirect effects), *Model 1* was run considering rosette diameter scored on tester plants in the three intergenotype treatments.

- *Estimating broad sense heritability values*

Based on variance components estimated by the REML method (PROC VARCOMP procedure with SAS 9.3, SAS Institute Inc.), the broad sense heritability value of the measured phenotypic trait ( $H^2_{\text{trait}}$ ) was estimated for each treatment using the following model:

$$\text{Model 2: } Y_{il} = \mu_{\text{trait}} + \text{block}_i + \text{accession}_l + \epsilon_{il}$$

$$\text{such that } H^2_{\text{trait}} = V_F / (V_F + (V_R / n))$$

where " $V_F$ " is the component of the estimated variance between accessions, " $V_R$ " is the residual variance, and "n" is the mean number of replicates per accession.

The significance of  $H^2_{\text{trait}}$  was evaluated by testing the significance of the term "accession<sub>l</sub>" by fitting *Model 2* using the PROC MIXED procedure with SAS 9.3 (REML method).

## Chapter 2

- *Estimating genotypic values of accessions*

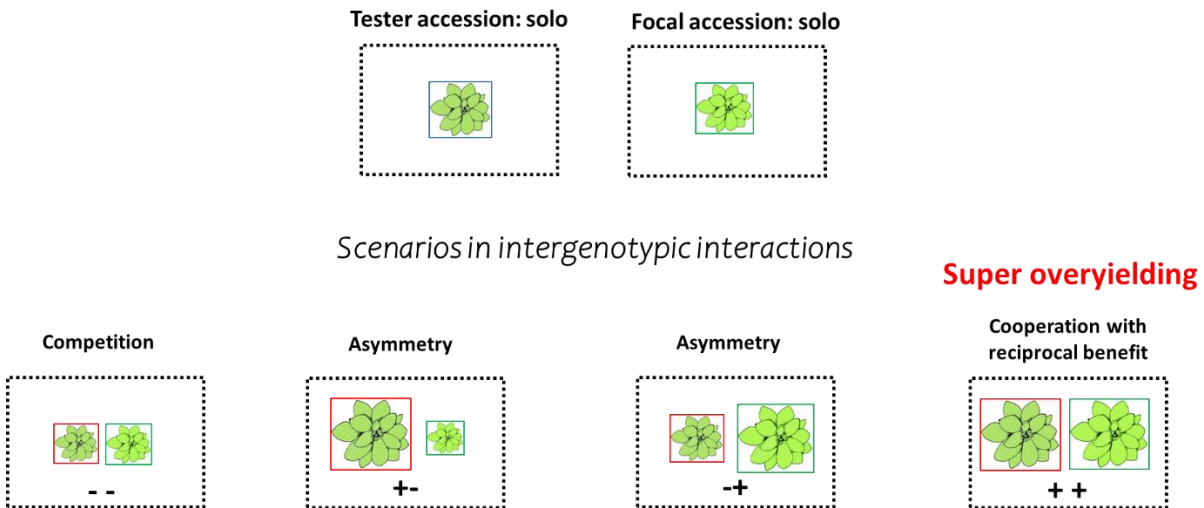
For each 'treatment \* accession' combination, the least squares means (LSmeans) of the rosette diameter were obtained independently on focal and tester plants using the following model (PROC GLM procedure, SAS 9.3, SAS Institute Inc.) :

$$\text{Model 3: } Y_{ijklm} = \mu_{\text{trait}} + \text{bloc}_i + \text{accession}_j + \text{germ}_m + \varepsilon_{ijklm}$$

Based on these genotypic values, relative genotypic values were calculated for the three tester accessions and the 192 focal accessions in each 'intergenotype' treatment as following:

$$\text{relative genotypic value} = \frac{(\text{genotypic value in intergenotype treatment} - \text{genotypic value in solo})}{\text{genotypic value in solo}}$$

As depicted in Figure 3, for each 'intergenotype' treatment, the relative genotypic values were then used to classify the intergenotypic interactions between a given tester and focal accessions in four broad categories (--, +-, +-, and ++).



**Figure 3: Illustration of the different outcomes of intergenotypic interactions between tester and focal accessions in our study.** Here "--" represents a reduction in the rosette diameter of tester accessions and focal accessions (compared to the diameters obtained when they grew alone), which results from competitive interactions. "+ -" represents the times where the 'tester accession - focal accession' interaction was positive for the tester accession and negative for the focal accession, and "- +" represents the reverse, both of which correspond to asymmetric interactions (altruism, Table 1). "++" represents an increase in rosette diameter of both partners (compared to the diameters obtained when they grew alone), suggesting cooperation with reciprocal benefit, *i.e.* a 'super overyielding' strategy.

### III.E. GWA mapping combined with a local score analysis

For each tester accession, in order to identify the genomic regions associated with the ‘super overyielding ++’ strategy, a GWA mapping approach was based on recoding the accessions with a ‘++’ strategy as 1 and the other accessions as 0 (‘other’ category). GWA mapping was run using a mixed model approach implemented in the EMMAX software (Efficient Mixed-Model Association Expedited; Kang *et al.* 2010). To control for the effect of population structure, we included as a covariate an identity-by-state kinship matrix  $K$  based on the 1,902,592 SNPs identified in the TOU-A population (Frachon *et al.* 2017). Moreover, because rare alleles increase the rate of false positives when included in mixed models (Brachi *et al.* 2010, Kang *et al.* 2010), we considered a threshold of minor allele relative frequency (MARF)  $> 7\%$  and ended up with 981,827 SNPs. In order to increase the probability to discover minor effect QTLs associated with phenotypic variation, as advocated in the previous chapter, we implemented a local score approach on the set of  $p$ -values provided by EMMAX (Bonhomme *et al.* 2019).

In order to compare the differences between the genetic architecture of cooperative interactions and the genetic architecture of competitive interactions, the same analysis was conducted by recoding the accessions with a ‘- -’ strategy as 1 and the other accessions as 0.

### III.F. Estimating genetic relationships underlying the ‘super overyielding’ strategy

For testing the ‘kin selection’ hypothesis, based on the whole genomic information, we expected that a specific tester accession has on average a higher kinship coefficient with the accessions cooperating with it than with the accessions belonging to the three other categories. On the other hand, testing the two other hypotheses involves estimating kinship coefficients based on the top SNPs associated with the ‘super overyielding’ strategy. For the ‘greenbeard effect’ hypothesis, we expected that a specific tester accession has on average a higher kinship coefficient with the accessions cooperating with it than with the accessions belonging to the three other categories, whereas the opposite outcome is predicted for the ‘compatibility genes’ hypothesis.

Kinship coefficients for testing each hypothesis were calculated based on either all SNPs (for testing the ‘kin selection’ hypothesis) or top SNPs associated with the ‘++’ strategy, by calculating identity by state (IBS) for each pair of accessions using the `snpGdsIBS` function in the *R* environment. We then ran an ANOVA for testing differences of kinship coefficients between the accessions having a ‘++’ strategy with the tester accession and the other accessions not having a ‘++’ strategy with the tester accession.

To further corroborate the outcomes of genetic relationships underlying positive interactions, we wanted to compare it with the patterns of genetic relationships underlying competitive interactions. Therefore, the same analysis was performed based on calculating kinship coefficients using the top SNPs associated with the ‘--’ strategy.

## IV. Results

### IV.A. Natural genetic variation of GxG interactions within a local population

- *Direct effects of the three tester accessions on focal accessions*

Rosette diameter of focal accessions were bigger in absence than in presence of a tester accession, with the mean diameter of plants growing in the ‘solo’ treatment being 19% bigger than the mean diameter of plants growing in the intergenotypic treatments (Table 2, Figure 3, Supplementary Figure 2). Interestingly, we detected a significant ‘generation’ effect that was not specific to a given treatment (Table 2), with accessions collected in 2010 being 14% smaller than accessions collected in 2002 (Figure 3).

We also detected a highly significant ‘accession’ effect, indicating strong genetic variation among focal accessions for rosette diameter within the TOU-A population (Table 2, Figure 4). Accordingly, significant high broad-sense heritability values ranging from 0.72 to 0.78 were found across the four treatments (Figure 4). More importantly, we detected significant ‘treatment \* accession’ interactions (Table 2), resulting in a pattern of crossing reaction norms among the four treatments (Figure 4). In other words, the response of the focal accessions varied not only

## Chapter 2

according to the presence or absence of a tester accession, but also according to the identity of the tester accession.

### A

Effect	Num DF	Den DF	<i>F</i> value	<i>P</i>
<b>Block</b>	<b>2</b>	<b>5.89</b>	<b>22.07</b>	<b>0.0018</b>
<b>Treatment</b>	<b>3</b>	<b>6.26</b>	<b>28.76</b>	<b>0.0005</b>
<b>Generation</b>	<b>1</b>	<b>894</b>	<b>149.05</b>	<b>&lt;.0001</b>
<b>Accession(Generation)</b>	<b>176</b>	<b>894</b>	<b>8.49</b>	<b>&lt;.0001</b>
Treatment*Generation	3	893	0.91	0.434
<b>Treatment*Accession(Generation)</b>	<b>502</b>	<b>894</b>	<b>1.29</b>	<b>0.0005</b>
<b>Germ</b>	<b>1</b>	<b>897</b>	<b>86.31</b>	<b>&lt;.0001</b>

### B

Effect	Num DF	Den DF	<i>F</i> value	<i>P</i>
<b>Block</b>	<b>2</b>	<b>3.7</b>	<b>19.09</b>	<b>0.0112</b>
Treatment	2	3.93	0.57	0.6052
<b>Generation</b>	<b>1</b>	<b>617</b>	<b>94.8</b>	<b>&lt;.0001</b>
<b>Accession(Generation)</b>	<b>176</b>	<b>618</b>	<b>6.46</b>	<b>&lt;.0001</b>
Treatment*Generation	2	617	1.19	0.3041
<b>Treatment*Accession(Generation)</b>	<b>326</b>	<b>618</b>	<b>1.2</b>	<b>0.0265</b>
<b>Germ</b>	<b>1</b>	<b>620</b>	<b>67.97</b>	<b>&lt;.0001</b>

**Table 2: Natural variation of local intraspecific GxG interactions based on rosette diameter measured on focal plants.** A) Considering the four treatments. B) Considering only the three intergenotype treatments. The terms ‘DF num’ and ‘DF den’ refer to the degrees of freedom of numerator and denominator, respectively. *F*-value: value of ratio of between-group variance on within-group variance. *P*: *p*-value associated with *F*-values. Significant effects are in bold.

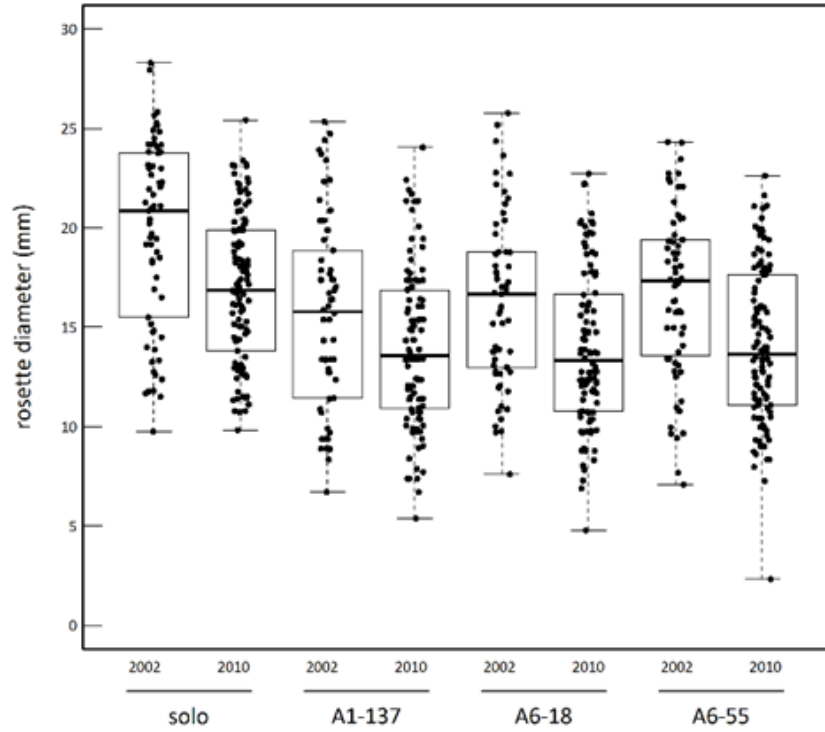


Figure 3: Boxplots illustrating genotypic values (LSmeans) of rosette diameter of focal accessions collected in 2002 and 2010 in absence and presence of each of the three testers. Each dot corresponds to one accession.

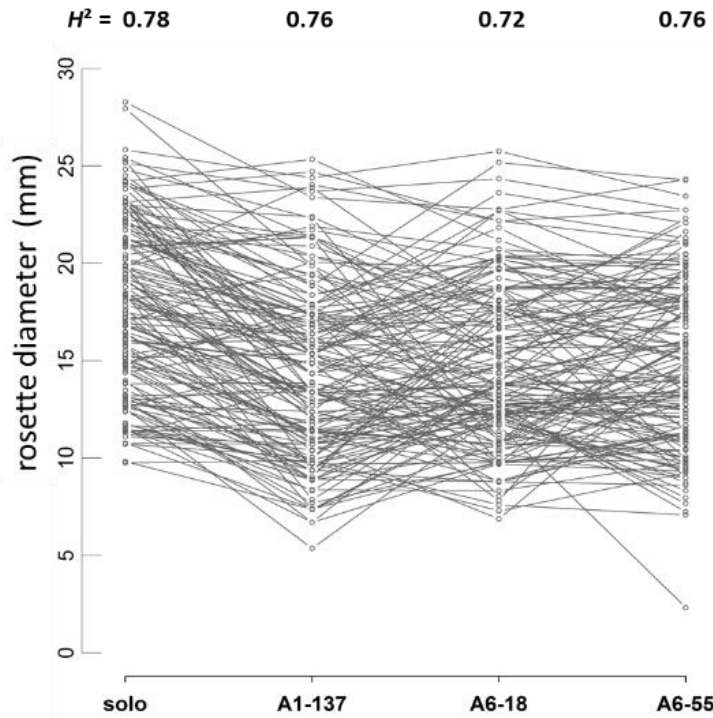


Figure 4: Interaction plot illustrating the genetic variation of direct effects among focal accessions in presence of each of the three testers. Each line connects the response of a specific focal accession across the four treatments. Broad sense heritability values ( $H^2$ ) are indicated on the top for each treatment.

## Chapter 2

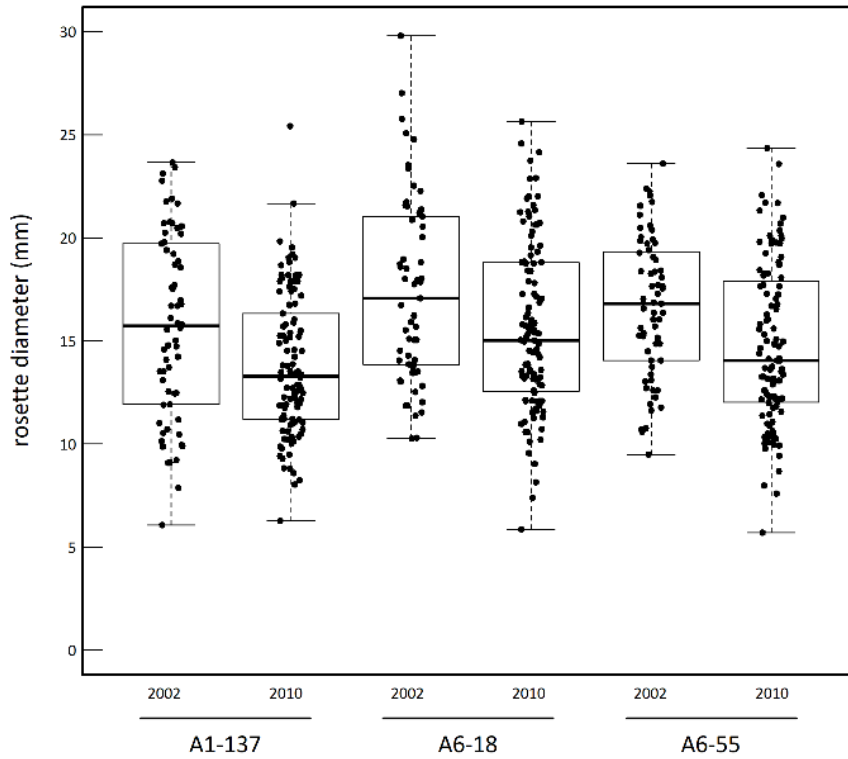
- *Indirect effects of focal accessions on testers*

We detected a highly significant ‘treatment’ effect on the rosette diameter of the tester plants (Table 3), with the plants from the tester accession A1-137 being on average smaller than the plants from the tester accessions A6-18 and A6-55 when grown in presence of focal accessions (Figure 5, Supplementary Figure 3). As for the rosette diameter scored on the focal plants, we detected a highly significant ‘generation’ effect for the rosette diameter scored on the tester plants (Table 3), with rosette diameter of the tester plants being on average 12% smaller in presence of accessions collected in 2010 than in presence of accessions collected in 2002 (Figure 5).

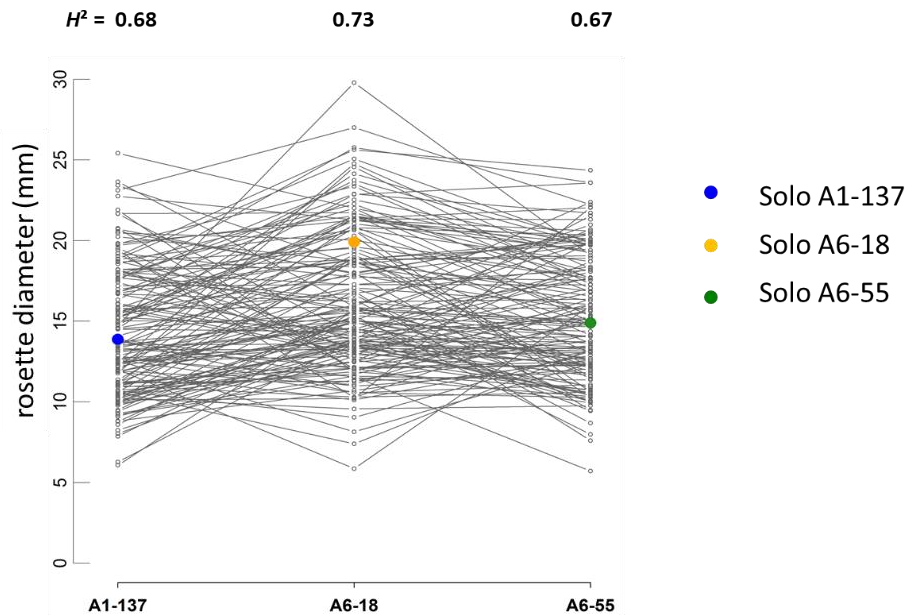
Effect	Num DF	Den DF	F value	<i>P</i>
<b>Block</b>	<b>2</b>	<b>614</b>	<b>45.13</b>	<b>&lt;.0001</b>
<b>Treatment</b>	<b>2</b>	<b>614</b>	<b>13.07</b>	<b>&lt;.0001</b>
<b>Generation</b>	<b>1</b>	<b>614</b>	<b>55.92</b>	<b>&lt;.0001</b>
<b>Accession(Generation)</b>	<b>176</b>	<b>614</b>	<b>4.9</b>	<b>&lt;.0001</b>
Treatment*Generation	2	614	0.41	0.6663
<b>Treatment*Accession(Generation)</b>	<b>326</b>	<b>614</b>	<b>1.45</b>	<b>&lt;.0001</b>
<b>Germ</b>	<b>1</b>	<b>614</b>	<b>13.02</b>	<b>0.0003</b>
<b>Diff</b>	<b>6</b>	<b>614</b>	<b>2.56</b>	<b>0.0188</b>

**Table 3: Natural variation of local intraspecific GxG interactions based on rosette diameter measured on tester plants.** The terms ‘DF num’ and ‘DF den’ refer to the degrees of freedom of numerator and denominator, respectively. *F*-value: value of ratio of between-group variance on within-group variance. *P*: *p*-value associated with *F*-values. Significant effects are in bold.

We detected a highly significant ‘accession’ effect (Table 3), suggesting strong genetic variation among the focal accessions on their effect on rosette diameter of the tester accessions. Accordingly, significant high broad-sense heritability values ranging from 0.67 to 0.73 were found across the three intergenotype treatments (Figure 6). More importantly, we detected significant ‘treatment \* accession’ interactions (Table 3), resulting in a pattern of crossing reaction norms among the three intergenotype treatments (Figure 6). In other words, differences in the response among the three testers to a given focal accession were highly dependent on the identity of the focal accessions.



**Figure 5: Boxplots illustrating genotypic values (LSmeans) of rosette diameter of tester accessions in presence of the focal accessions collected in 2002 and 2010.** Each dot corresponds to the effect of one focal accession on a given tester accession.



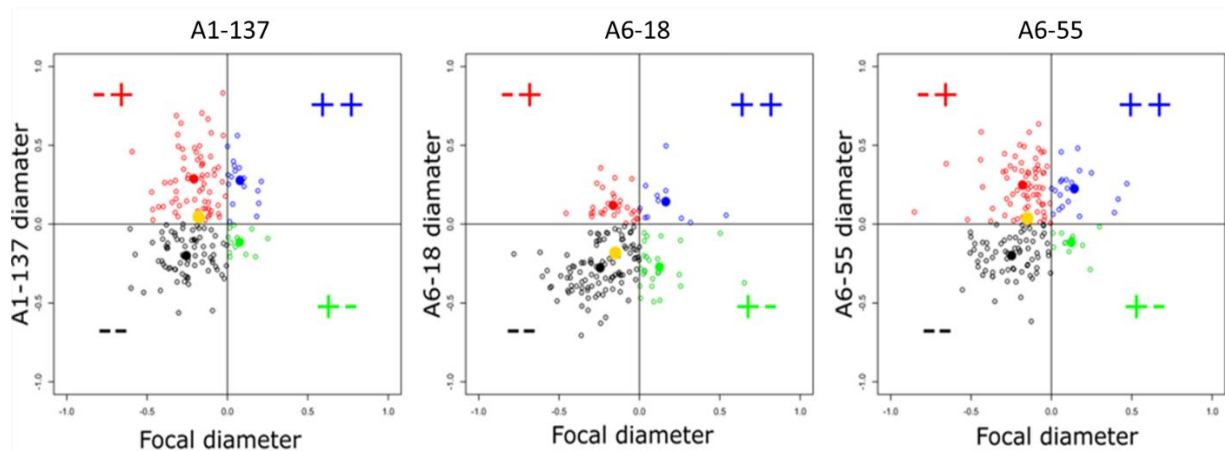
**Figure 6: Interaction plot illustrating the genetic variation of indirect effects among the focal accessions on each of the three testers.** Each line connect the effect of a specific focal accession on the three testers. Colored dots indicate the genotypic values of the three testers when grown in the ‘solo’ treatment. Broad sense heritability values ( $H^2$ ) are indicated on the top for each treatment.

## Chapter 2

- *Outcomes of GxG interactions within the TOU-A population*

By combining the response of both focal and tester accessions (compared to the diameters obtained when they grew alone), we classified each combination of accession in four categories, *i.e.* competitive interactions ('--'), asymmetric interactions ('+-' and '-+') and cooperative interactions ('++' or 'super overyielding' strategy). These four categories of interactions were identified for each tester, but with varying proportions depending on the identity of the tester accession (Figure 7).

Competitive interactions was the most prevalent strategy, with 41.5% to 62.1% of intergenotypic interactions corresponding to a situation where both partners suffer a decrease in rosette diameter. Interestingly, a 'super overyielding' strategy was observed for a significant portion of intergenotypic interactions, between 6.5% and 11.7% depending on the tester accession (Table 4). In addition, only two cooperating accessions were common between the three tester accessions, suggesting that the genetics underlying the 'super overyielding' strategy within this population can be highly dependent on the identity of the neighbouring plant.



**Figure 7: Plot depicting four different interaction categories between the focal accessions and the three testers (A1-137, A6-18 and A6-55).** The x-axis represents rosette diameter of the focal accessions in presence of a given tester relative to the corresponding rosette diameter observed in the 'solo' treatment. The y-axis represents rosette diameter of the tester accessions in presence of the focal accessions relative to the corresponding rosette diameter observed in the 'solo' treatment. '++' represents cooperation ('super overyielding strategy'), *i.e.* when both the interacting partners showed an increase in rosette diameter. '-+' and '+-' show altruism, *i.e.* one of the partners shows an increase in rosette diameter at the expense of its partner accession. '-+' is when focal plant is suffering a decrease in rosette diameter and '+-' is when the tester shows a decrease in rosette diameter. '--' indicates competition where both partners suffer a decrease in rosette diameter.

Interaction type	A1-137	A6-18	A6-55
--	43.5%	62.1%	41.5%
+-	39.3%	16.6%	40.4%
-+	7.1%	14.8%	6.4%
++	10.1%	6.5%	11.7%

**Table 4: Proportion of the different types of interactions for each tester accession.**

## IV.B. Genetic architecture of positive GxG interactions

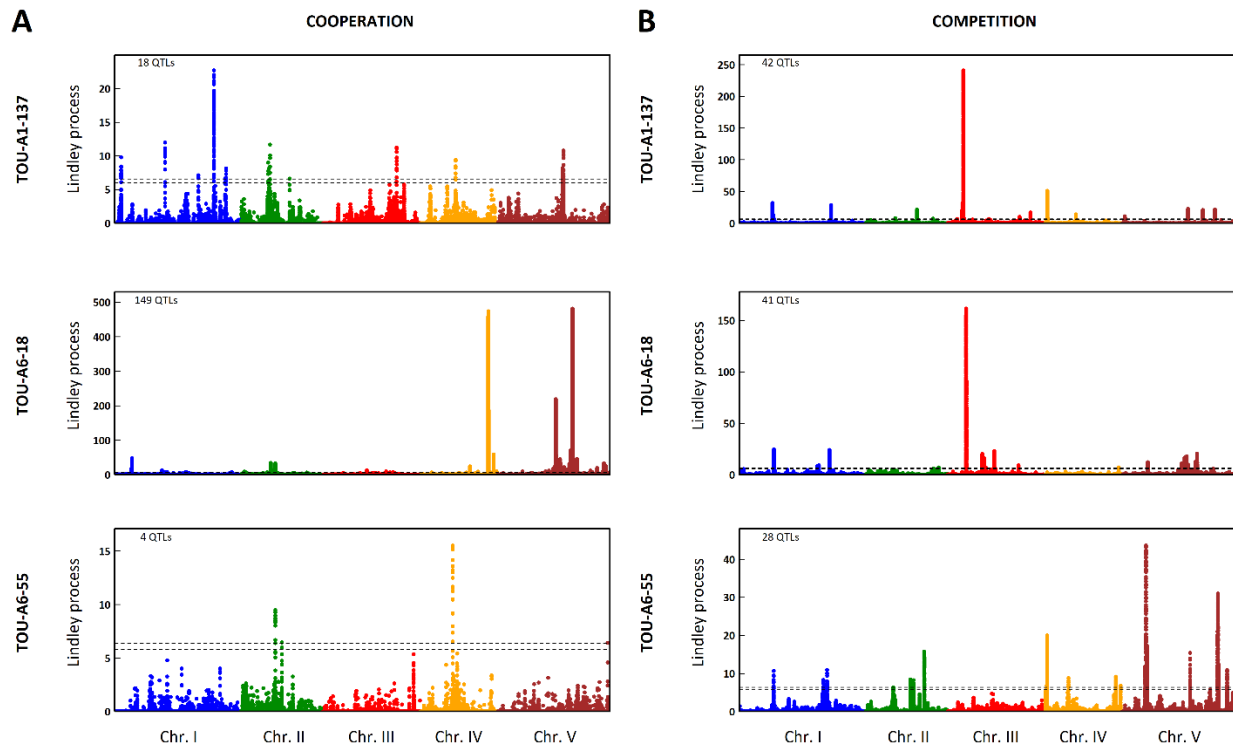
Combining GWA mapping with a genome-wide local score analysis revealed for each tester accession, neat and strong association peaks for the ‘super overyielding’ strategy (Figure 8A). However, we observed a contrasted genetic architecture between the three tester accessions. We detected a relatively small number of QTLs with the tester accessions A1-137 and A6-55 in comparison with the tester accession A6-18 (Figure 8A). For the tester accession A1-137, we detected 18 QTLs associated with the ‘super overyielding’ strategy, each supported on average by ~13 significant SNPs (min = 3, max = 53). In agreement with the short linkage disequilibrium observed in the TOU-A population (Frachon *et al.* 2019), the mean QTL size was 0.95kb (min = 4bp, max = 3.87kb). For the tester accession A6-55, we detected four QTLs associated with the ‘super overyielding’ strategy, each supported on average by ~8 significant SNPs (min = 2, max = 18). The mean QTL size was 0.26kb (min = 40bp, max = 0.71kb).

By contrast, for the tester accession A6-18, we detected 149 QTLs associated with the ‘super overyielding’ strategy, with 65% of them being located at the end of chromosome 5 (Figure 8A). These observations for the tester accession A6-18 suggest an enrichment in low  $p$ -values generated by the mixed model implemented in the EMMA software, probably resulting from the low number of accessions expressing a ‘super overyielding’ strategy with this tester accession (Figure 7, Table 4). We therefore discarded the GWA mapping results obtained for the tester accession A6-18.

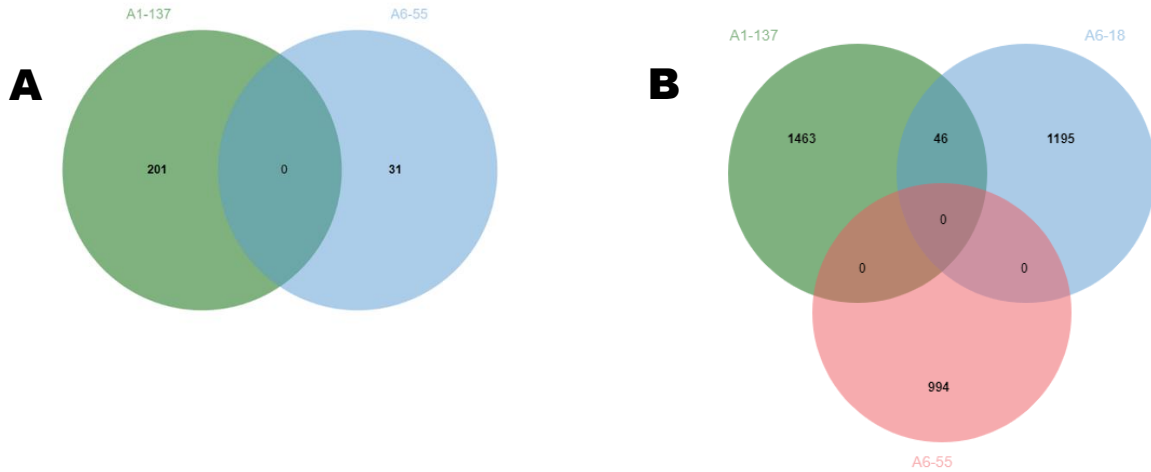
## Chapter 2

In agreement with the very small number of cooperating accessions common between the tester accessions A1-137 and A6-55, none of the top SNPs identified were shared among these two testers (Figure 9A).

The genetic architecture of competitive interactions was more polygenic than the genetic architecture of cooperative interactions, with the number of QTLs detected for competitive interactions ranging from 28 to 42 (Figure 8B). As previously observed for the ‘super overyielding’ strategy, the genetic architecture of competitive interactions was highly dependent on the identity of the tester accession, with a very small portion of top SNPs shared between the three tester accessions (Figure 9B).



**Figure 8: Genomic maps of cooperative and competitive GxG interactions.** A) Manhattan plot of Lindley process values (local score method with  $\xi = 2$ ) for cooperation with reciprocal benefit (++) for each of the three testers (A1-137, A6-18 and A6-55). B) Manhattan plot of Lindley process values (local score method with  $\xi = 2$ ) for competitive interactions (--) for each of the three testers (A1-137, A6-18 and A6-55). The x-axis corresponds to the physical position of 981,827 SNPs on the five chromosomes. The y-axis designates the value of the Lindley process. The dashed line indicates the significance threshold. The number of QTLs detected by applying a local score approach is indicated on the top left corner.



**Figure 9: The genetic architecture of GxG interactions is highly dependent on the identity of the tester accession.** A) Venn diagram comparing the top SNPs associated with the ‘super overyielding’ strategy between the two tester accessions A1-137 and A6-55, and B) Venn diagram comparing the top SNPs associated with competition among the three tester accessions.

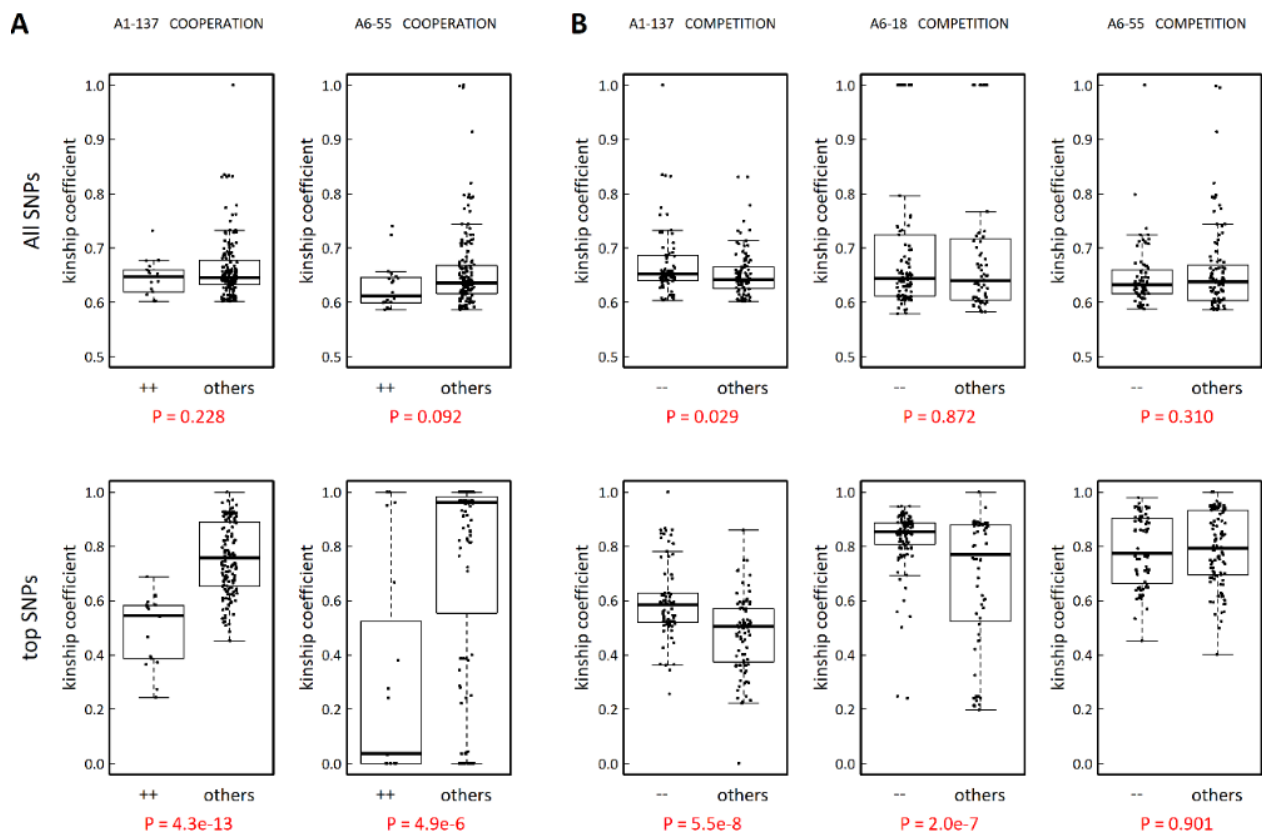
#### IV.C. Investigating relationships between cooperators at the genomic level

To test the ‘kin selection’ hypothesis, we first calculated the kinship coefficient between each of the focal accessions and each of the two testers A1-137 and A6-55, based on the whole set of SNPs. For each tester accession, the kinship coefficients were similar between the set of cooperating focal accessions and the set of focal accessions belonging to the other three interaction categories (Figure 10A). We applied the same procedure by differentiating the set of competitive focal accessions from the set of accessions belonging to the other three interaction categories. At the whole genome level, the kinship coefficients were also similar between these two sets of accessions (Figure 10B).

In a second step, to test for the ‘greenbeard effect’ and ‘compatibility genes’ hypotheses, we calculated kinship coefficient between each of the focal accessions and each of the two tester accessions A1-137 and A6-55, based on the top SNPs identified by GWA mapping (Figure 8A). For each tester accession, the kinship coefficient of the cooperating accessions was on average significantly smaller than the kinship coefficient of the other focal accessions belonging to the

## Chapter 2

three other interaction categories (Figure 10A). By contrast, for the tester accessions A1-137 and A6-18 but not for the tester accession A6-55, the kinship coefficient of the competing accessions was on average significantly higher than the kinship coefficient of the focal accessions belonging to the three other interaction categories (Figure 10B). Overall, these results suggest that the ‘compatibility genes’ strategy underlies the ‘super overyielding’ strategy observed in the TOU-A population.



**Figure 10: Comparing different hypotheses for genetic relationships between A) cooperating (++) and B) competing (--) accessions with each tester accession, based on kinship coefficients estimated at the whole genome level (top panels) or based on top SNPs (bottom panels). In each panel, each dot of the box located on the left corresponds to one accession either cooperating or competing with the tester accession, whereas each dot of the box located on the right corresponds to one accession belonging to any of the three other categories (‘others’). For each panel, differences of kinship coefficients between the two types of interactions (‘++’ vs ‘others’, ‘--’ vs ‘others’) were tested by running an ANOVA. Resulting *p*-values are indicated under each panel.**

#### IV.D. Genetic bases associated with the ‘super overyielding’ strategy

To identify candidate genes associated with the ‘++’ strategy among the tester accessions A1-137 and A6-55, we retrieved 37 annotated genes located within or overlapping with the detected 22 QTL regions (Supplementary Table 2). On the basis of the annotations and bibliography available, a large proportion of the functions encoded by these genes are related to signaling and/or regulatory processes (including transcription factors) (10 genes, 27%). Another large chunk of these genes encodes proteins of unknown functions (29%). Among the remaining genes, four genes have been found encoding proteins putatively involved in cell wall biosynthesis: a pectin lyase-like superfamily protein (AT1G70500), a glycin rich protein (AT2G15340), an invertase/pectin methylesterase inhibitor superfamily protein (AT2G15345) and a hydroxyproline-rich glycoprotein family protein (AT2G18910). In summary, whatever the tester used in our experiments, the candidate genes associated with the QTLs identified for the ‘++’ strategy encode various functions, with a significant proportion of signaling-related functions.

### V. Discussion and perspectives

Despite extensive demonstration of positive interactions within wild plant populations in multiple studies, the underlying genetic architecture is still largely unknown (Subrahmaniam *et al.* submitted). Here, by combining quantitative genetics and association genetics, we aimed at understanding the genetics associated with a ‘super overyielding’ strategy within a local population of *A. thaliana*.

#### V.A. The occurrence of a ‘super overyielding’ strategy in the local TOU-A population

Interestingly, we identified a small but significant fraction of intergenotypic combinations presenting a ‘super overyielding’ strategy, indicating that some accessions performed even better when growing with another accession than when growing alone. This could imply that these

## Chapter 2

accessions may be in need to form cooperative links to derive mutual benefits available, which would not be possible otherwise. Presence of these cooperative links can potentially explain the maintenance of a high level of genetic diversity observed in the TOU-A population, despite its highly competitive environment (Frachon *et al.* 2017).

As demonstrated in this experiment, ‘super overyielding’ combinations were largely distinct among the three testers, suggesting the presence of diffuse cooperative links among genotypes present within this local population. This could be based upon the probabilities of natural interactions occurring among genotypes. Not all genotypes can interact regularly in a given population. Therefore, based on their probabilities of regular interactions in nature, the genetic architecture of positive interactions could vary between different cooperating pairs.

Altogether, these results reinforce the need to include intraspecific genetic variation for understanding eco-evolutionary patterns of natural plant communities (Hughes *et al.* 2008). However, full extents of these diffuse cooperative links cannot be estimated in this experiment as only three tester accessions were used and they were occupying extreme corners of the genomic space. Therefore, a step in the future direction would be to pick up random accessions (between 10 and 20) from this local population and to test all pairwise combinations. In addition, we advocate adding an ‘intra-genotype’ treatment for future experiments to estimate behavior of the TOU-A accessions in the presence of a kin *vs* non-kin from the same population. Furthermore, the outcome of GxG interaction patterns may vary depending on the trait measured. Therefore, to get a full picture of the plant’s condition in GxG interactions and the dynamics associated with intraspecific positive interactions (as demonstrated in the first experimental chapter), we advocate measuring multiple phenotypic traits and/or the same phenotypic trait at multiple time points.

### **V.B. The ‘super overyielding’ strategy observed in the TOU-A population may be driven by compatibility genes**

We tested three hypotheses for exploring the genetic relationships underling the ‘super overyielding’ strategy observed in this experiment. We did not confirm the ‘kin selection’ or ‘greenbeard effect’ hypotheses. Instead, the ‘compatibility genes’ hypothesis was confirmed by

## Chapter 2

the identification of cooperating accessions having on average different alleles at QTLs associated with the ‘super overyielding’ strategy.

One explanation of an ‘over yielding’ strategy in some genotypic mixtures can be related to the observation of metabolite cross-feeding in microbial interactions (D’Souza *et al.* 2018). Such mutual cross-feeding has been postulated to be favored by low environmental resource availabilities, by selecting for greater resource exchange in the face of adverse conditions (Smith *et al.* 2019). Whether our ‘super overyielders’ also exchange metabolites is an open question. If so, whether metabolite exchange is direct (*i.e.* soil diffusion) or indirect (*i.e.* through soil or rhizosphere microbiota) is an additional question.

As a first step to test for beneficial exchanges of metabolites between a given tester accession and cooperating accessions, we might explore the root exudate profile (primary and secondary metabolites) characterized on all the 195 TOU-A accessions by our colleague Catherine Rameau (INRA, Versailles). In particular, we can test whether cooperating accessions have more complementary exudate profiles than competing accessions. We can also consider characterizing the root exudate profile of our tester accessions in absence and in presence of cooperating focal accessions.

### V.C. Candidate genes underlying the ‘super overyielding’ strategy

While our work in the previous chapter (field experiment) revealed that metabolic functions might be essential for cooperation, here a significant proportion of the candidate genes found encode functions related to signaling and regulatory processes, including transcription factors. Surprisingly, another large part of the candidate genes encodes proteins of unknown functions, suggesting that plant cooperation may involve functions not yet recruited for other biotic interactions, or more widely for plant development. However, a few of them show interesting biological functions in the context of plant cooperation, and some of them are involved in regulatory functions associated in some cases to metabolism (as described below).

KNAT2 (KNOTTED-LIKE FROM ARABIDOPSIS THALIANA 2, AT1G70510) is a transcription factor of the knotted1-like homeobox gene family, which has been shown to control

## Chapter 2

shoot apical and floral meristems (Li *et al.* 2011, Zhao *et al.* 2015, Lozano-Sotomayor *et al.* 2016). Moreover, a large body of evidence suggests that KNOX transcriptional activity is essential for the determination of organ versus meristem identity, and regulates the production of gibberellins. Taken together, these findings indicate that KNAT2 acts as a player in the complex network of transcription factors controlling meristematic activities in the plant (Kuijt *et al.* 2014), which might be important to regulate plant growth under cooperative interactions.

Another developmental function of putatively high interest for cooperative interactions, is LPR2 (AT1G71040), a multicopper oxidase, which mediates with its close homolog LPR1, phosphate sensing at the root tip, and the developmental response of root meristems to phosphate availability (Ticconi *et al.* 2009). Partners in plant-plant interactions might influence soil conditions for each other and it is known that remodeling of root architecture to fluctuating soil conditions is accomplished by adjustment of lateral root formation and root growth (more lateral roots and higher density of roots in Pi deficiency) under the control of LPR1 and LPR2 (Muller *et al.* 2015). In addition, a recent study revealed that LPR1 and 2 drastically influence the nature of root exudate metabolites during the local Pi deficiency response (Ziegler *et al.* 2015). Together, these data suggest a central role for LPR1 and 2 in monitoring not only nutrient availability, but also metabolite production.

Finally, CNGC16 is a member of the cyclic nucleotide-gated ion channel (CNGCs) family that are potential cation transporters involved in the plant response to biotic/abiotic stresses (Balagué *et al.* 2003, Defalco *et al.* 2016), growth and development and ion homeostasis (Moon *et al.* 2019). Due to their potential non-selectivity in the uptake of different cations, CNGCs are also involved in the uptake of micronutrient ions into cells, as well as toxic ions (Gobert *et al.* 2006).

These functions might be key actors in the context of plant-plant interactions, especially CNGC16 and LPR2, which might exert functions in good agreement with our previous finding showing preeminence of metabolic functions underlying QTLs associated with OY/KC variation among a set of 52 regional populations of *A. thaliana*.

## VI. References

- Balagué, C., Lin, B., Alcon, C., Flottes, G., Malmström, S., Köhler, C., Neuhaus, G., Pelletier, G., Gaymard, F. & Roby, D. (2003). HLM1, an essential signaling component in the hypersensitive response, is a member of the cyclic nucleotide-gated channel ion channel family. *The Plant Cell*, 15, 365–379. <https://doi.org/10.1105/tpc.006999.2>
- Baron, E., Richirt, J., Villoutreix, R., Amsellem, L., & Roux, F. (2015). The genetics of intra- and interspecific competitive response and effect in a local population of an annual plant species. *Functional Ecology*, 29(10), 1361–1370. <https://doi.org/10.1111/1365-2435.12436>
- Bonhomme, M., Fariello, M. I., Navier, H., Hajri, A., Badis, Y., Miteul, H., Samac, D.A., Dumas, B., Baranger, A., Jacquet, C. & Pilet-Nayel, M. L. (2019). A local score approach improves GWAS resolution and detects minor QTL: application to *Medicago truncatula* quantitative disease resistance to multiple *Aphanomyces euteiches* isolates. *Heredity*, 123(4), 517–531. <https://doi.org/10.1038/s41437-019-0235-x>
- Brachi, B., Faure, N., Horton, M., Flahauw, E., Vazquez, A., Nordborg, M., Bergelson, J., Cuguen, J., & Roux, F. (2010). Linkage and association mapping of *Arabidopsis thaliana* flowering time in nature. *PLoS Genetics*, 6(5), e1000940. <https://doi.org/10.1371/journal.pgen.1000940>
- Brachi, B., Faure, N., Bergelson, J., Cuguen, J. & Roux, F. (2013). Genome-wide association mapping of flowering time in *Arabidopsis thaliana* in nature: genetics for underlying components and reaction norms across two successive years. *Acta Bot Gallica*, 2013;160(3-4):205. doi:10.1080/12538078.2013.807302
- Brooker, R. W. (2006). Plant – plant interactions and environmental change. *New Phytologist*, 171(2), 271–284. <https://doi.org/10.1111/j.1469-8137.2006.01752.x>
- Bruno, J. F., Stachowicz, J. J. & Bertness, M. D. (2003). Inclusion of facilitation into ecological theory. *Trends in Ecology and Evolution*, 18(3), 119–125. [https://doi.org/10.1016/S0169-5347\(02\)00045-9](https://doi.org/10.1016/S0169-5347(02)00045-9)
- Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics*, 31, 343–366. <https://doi.org/10.1146/annurev.ecolsys.31.1.343>
- Debieu, M., Huard-Chauveau, C., Genissel, A., Roux, F. & Roby, D. (2016). Quantitative disease resistance to the bacterial pathogen *Xanthomonas campestris* involves an Arabidopsis immune receptor pair and a gene of unknown function. *Molecular Plant Pathology*, 17, 510–520.
- DeFalco, T. A., Moeder, W. & Yoshioka, K. (2016). Opening the Gates: Insights into Cyclic Nucleotide-Gated Channel-Mediated Signaling. *Trends in Plant Science*, 21(11), 903–906. <https://doi.org/10.1016/j.tplants.2016.08.011>
- Dormann, C. F. & Brooker, R. W. (2002). Facilitation and competition in the high Arctic: The importance of the experimental approach. *Acta Oecologica*, 23(5), 297–301. [https://doi.org/10.1016/S1146-609X\(02\)01158-X](https://doi.org/10.1016/S1146-609X(02)01158-X)
- D’Souza, G., Shitut, S., Preussger, D., Yousif, G., Waschina, S. & Kost, C. (2018). Ecology and evolution of metabolic cross-feeding interactions in bacteria. *Natural Product Reports*, 35(5), 455–488. <https://doi.org/10.1039/c8np00009c>
- Ehlers, B. K., Damgaard, C. F., & Laroche, F. (2016). Intraspecific genetic variation and species coexistence in plant communities. *Biology Letters*, 12(1). <https://doi.org/10.1098/rsbl.2015.0853>
- Frachon, L., Libourel, C., Villoutreix, R., Carrère, S., Glorieux, C., Huard-Chauveau, C., Navascues, M., Gay, L., Vitalis, R., Baron, E., Amsellem, L., Bouchez, O., Vidal, M., Le Corre, V., Roby, D., Bergelson, J. & Roux, F. (2017). Intermediate degrees of synergistic pleiotropy drive adaptive evolution in ecological time. *Nature Ecology & Evolution*, 1, 1551–1561. <https://doi.org/10.1038/s41559-017-0297-1>

## Chapter 2

Gobert, A., Park, G., Amtmann, A., Sanders, D., & Maathuis, F. J. M. (2006). *Arabidopsis thaliana* Cyclic Nucleotide Gated Channel 3 forms a non-selective ion transporter involved in germination and cation transport. *Journal of Experimental Botany*, 57(4), 791–800. <https://doi.org/10.1093/jxb/erj064>

Goldberg, D. E. & Barton, A. M. (1992). Patterns and consequences of interspecific competition in natural communities - a review of field experiments with plants. *American Naturalist*, 139(4), 771–801. <https://doi.org/10.1086/285357>

Huard-Chauveau, C., Percepied, L., Debieu, M., Rivas, S., Kroj, T., Kars, I., Bergelson, J., Roux, F. & Roby, D. (2013). An Atypical Kinase under Balancing Selection Confers Broad Spectrum Disease Resistance in *Arabidopsis*. *PLoS Genetics* 9(9).doi:10.1371/journal.pgen.1003766

Hughes, A. R., Inouye, B. D., Johnson, M. T. J., Underwood, N. & Vellend, M. (2008). Ecological consequences of genetic diversity. *Ecology Letters*, 11(6), 609–623. <https://doi.org/10.1111/j.1461-0248.2008.01179.x>

Kuijt, S. J. H., Greco, R., Agalou, A., Shao, J., 't Hoen, C. C. J., Övernäs, E., Osnato, M., Curiale, S., Meynard, D., van Gulik, R., Maraschin, S.D.F., Atallah, M., de Kam, R.J., Lamers, G.E.M., Guiderdoni, E., Rossini, L., Meijer, A.H. & Ouwkerk, P. B. F. (2014). Interaction between the growth-regulating factor and knotted1-like homeobox Families of Transcription Factors. *Plant Physiology*, 164(4), 1952–1966. <https://doi.org/10.1104/pp.113.222836>

Li, Y., Pi, L., Huang, H., & Xu, L. (2012). *ATH1 and KNAT2 proteins act together in regulation of plant inflorescence architecture*. 63(3), 1423–1433. <https://doi.org/10.1093/jxb/err376>

Lozano-Sotomayor, P., Chávez Montes, R. A., Silvestre-Vañó, M., Herrera-Ubaldo, H., Greco, R., Pablo-Villa, J., Galliani, B.M., Diaz-Ramirez, D., Weemen, M., Boutilier, K., Pereira, A., Colombo, L., Madueno, F., Marsch-Martinez, N. & de Folter, S. (2016). Altered expression of the bZIP transcription factor DRINK ME affects growth and reproductive development in *Arabidopsis thaliana*. *Plant Journal*, 88(3), 437–451. <https://doi.org/10.1111/tbj.13264>

Moon, J. Y., Belloeil, C., Ianna, M. L. & Shin, R. (2019). *Arabidopsis* CNGC family members contribute to heavy metal ion uptake in plants. *International Journal of Molecular Sciences*, 20(2). <https://doi.org/10.3390/ijms20020413>

Müller, J., Toev, T., Heisters, M., Teller, J., Moore, K. L., Hause, G., Dinesh, D.H., Burstenbinder, K. & Abel, S. (2015). Iron-Dependent Callose Deposition Adjusts Root Meristem Maintenance to Phosphate Availability. *Developmental Cell*, 33(2), 216–230. <https://doi.org/10.1016/j.devcel.2015.02.007>

Platt, A., Horton, M., Huang, Y. S., Li, Y., Anastasio, A. E., Mulyati, N. W., Agren, J., Bossdorf, O., Byers, D., Donohue, K., Dunning, M., Holub, E. B., Hudson, A., Le Corre, V., Loudet, O., Roux, F., Warthmann, N., Weigel, D., Rivero, L., Scholl, R., Nordborg, M., Bergelson, J. & Borevitz, J. O. (2010). The scale of population structure in *Arabidopsis thaliana*. *PLoS Genetics*, 6(2). <https://doi.org/10.1371/journal.pgen.1000843>

Smith, N.W., Shorten, P.R., Altermann, E., Roy, N.C. & McNabb, W.C. (2019). The Classification and Evolution of Bacterial Cross-Feeding. *Frontiers in Ecology and Evolution*, 7:153. doi:10.3389/fevo.2019.00153

Subrahmaniam, H. J., Libourel, C., Journet, E. P., Morel, J. B., Muñoz, S., Niebel, A., Raffaele, S. & Roux, F. (2018). The genetics underlying natural variation of plant–plant interactions, a beloved but forgotten member of the family of biotic interactions. *The Plant Journal*, 93(4): 747-770.

Subrahmaniam, H. J., Roby, D. & Roux, F. (submitted to *Plant Communications*). Towards unifying evolutionary ecology and genomics to understand genotype-by-genotype interactions within wild plant species.

Ticconi, C. A., Lucero, R. D., Sakhonwasee, S., Adamson, A. W., Creff, A., Nussaume, L., Desnos, T. & Abel, S. (2009). ER-resident proteins PDR2 and LPR1 mediate the developmental response of root meristems to phosphate availability. *Proceedings of the National Academy of Sciences of the United States of America*, 106(33), 14174–14179. <https://doi.org/10.1073/pnas.0901778106>

## Chapter 2

Tilman, D., Lehman, C.L. & Thomson, K.T. (1997). Plant diversity and ecosystem productivity: theoretical considerations. *Proceedings of the National Academy of Sciences of the United States of America* 94:1857-1861.

Zhao, M., Yang, S., Chen, C. Y., Li, C., Shan, W., Lu, W., Cui, Y., Liu, X. & Wu, K. (2015). *Arabidopsis* BREVIPEDICELLUS interacts with the SWI2/SNF2 chromatin remodeling ATPase BRAHMA to Regulate KNAT2 and KNAT6 Expression in control of inflorescence architecture. *PLoS Genetics*, 11(3), 1–21. <https://doi.org/10.1371/journal.pgen.1005125>

Ziegler, J., Schmidt, S., Chutia, R., Müller, J., Böttcher, C., Strehmel, N., Scheel, D. & Abel, S. (2016). Non-targeted profiling of semi-polar metabolites in *Arabidopsis* root exudates uncovers a role for coumarin secretion and lignification during the local response to phosphate limitation. *Journal of Experimental Botany*, 67(5), 1421–1432. <https://doi.org/10.1093/jxb/erv539>

*VII. Supplementary information*

Chapter 2

**Supplementary Table 1:** List of the 195 TOU-A accessions (with their ecotype ID) collected in 2002 (TOU-A1) and 2010 (TOU-A6).

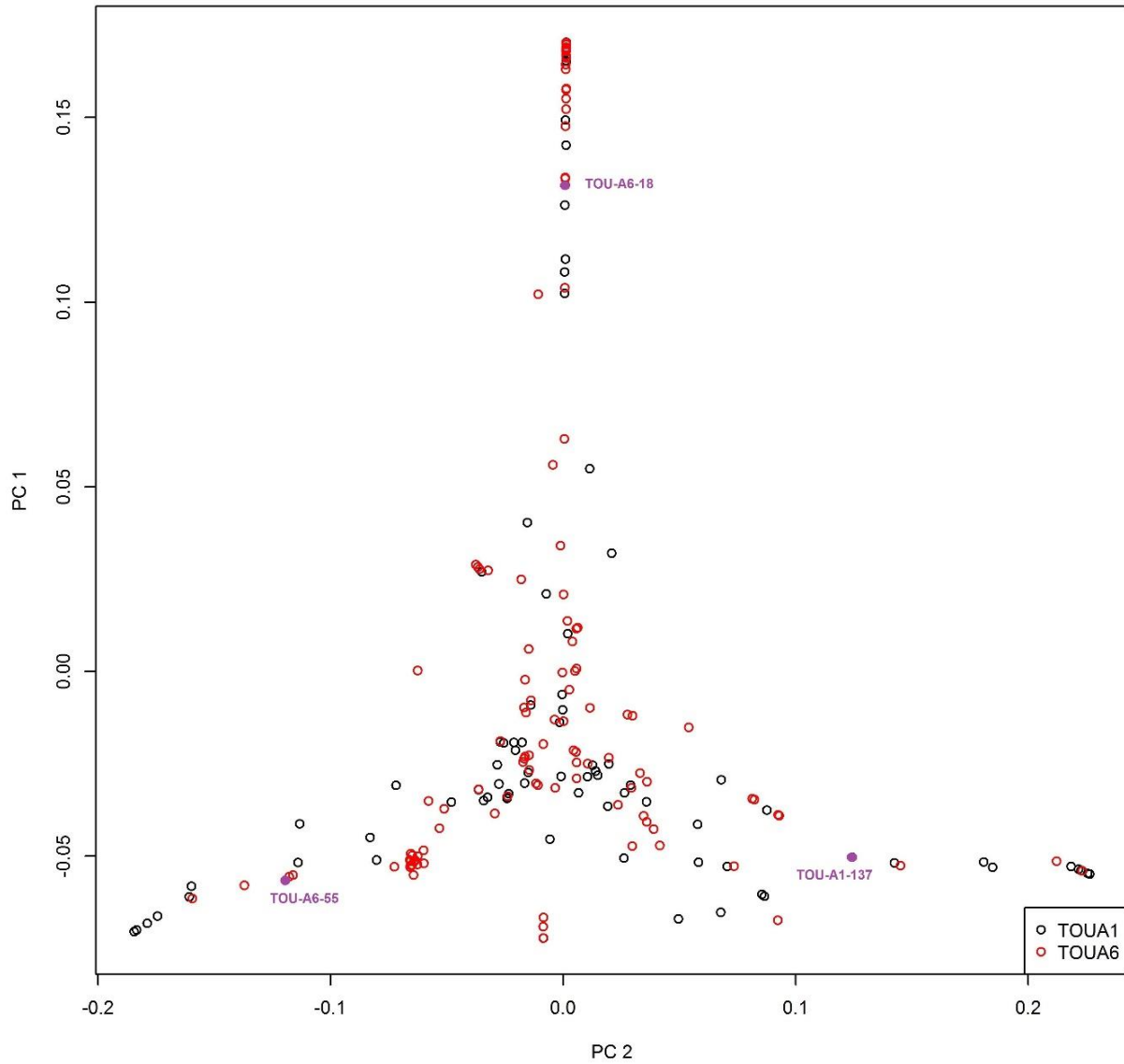
TOU-A1-10	71001	TOU-A1-116	71011	TOU-A1-129	71021	TOU-A1-15	71031
TOU-A1-105	71002	TOU-A1-117	71012	TOU-A1-13	71022	TOU-A1-16	71032
TOU-A1-106	71003	TOU-A1-118	71013	TOU-A1-131	71023	TOU-A1-17	71033
TOU-A1-109	71004	TOU-A1-119	71014	TOU-A1-133	71024	TOU-A1-18	71034
TOU-A1-110	71005	TOU-A1-12	71015	TOU-A1-134	71025	TOU-A1-19	71035
TOU-A1-111	71006	TOU-A1-120	71016	TOU-A1-137	71026	TOU-A1-2	71036
TOU-A1-112	71007	TOU-A1-122	71017	TOU-A1-138	71027	TOU-A1-23	71037
TOU-A1-113	71008	TOU-A1-124	71018	TOU-A1-14	71028	TOU-A1-26	71038
TOU-A1-114	71009	TOU-A1-125	71019	TOU-A1-141	71029	TOU-A1-27	71039
TOU-A1-115	71010	TOU-A1-128	71020	TOU-A1-143	71030	TOU-A1-29	71040
TOU-A1-3	71041	TOU-A1-6	71051	TOU-A1-73	71061	TOU-A1-84	71071
TOU-A1-33	71042	TOU-A1-60	71052	TOU-A1-74	71062	TOU-A1-85	71072
TOU-A1-34	71043	TOU-A1-61	71053	TOU-A1-75	71063	TOU-A1-88	71073
TOU-A1-37	71044	TOU-A1-62	71054	TOU-A1-77	71064	TOU-A1-89	71074
TOU-A1-38	71045	TOU-A1-63	71055	TOU-A1-78	71065	TOU-A1-9	71075
TOU-A1-39	71046	TOU-A1-65	71056	TOU-A1-79	71066	TOU-A1-90	71076
TOU-A1-4	71047	TOU-A1-66	71057	TOU-A1-8	71067	TOU-A1-91	71077
TOU-A1-41	71048	TOU-A1-67	71058	TOU-A1-80	71068	TOU-A1-93	71078
TOU-A1-43	71049	TOU-A1-69	71059	TOU-A1-81	71069	TOU-A1-96	71079
TOU-A1-47	71050	TOU-A1-7	71060	TOU-A1-82	71070	TOU-A1-98	71080
TOU-A6-1	71081	TOU-A6-108	71091	TOU-A6-117	71101	TOU-A6-2	71111
TOU-A6-10	71082	TOU-A6-109	71092	TOU-A6-118	71102	TOU-A6-20	71112
TOU-A6-100	71083	TOU-A6-11	71093	TOU-A6-12	71103	TOU-A6-21	71113
TOU-A6-101	71084	TOU-A6-110	71094	TOU-A6-13	71104	TOU-A6-22	71114
TOU-A6-102	71085	TOU-A6-111	71095	TOU-A6-14	71105	TOU-A6-23	71115
TOU-A6-103	71086	TOU-A6-112	71096	TOU-A6-15	71106	TOU-A6-24	71116
TOU-A6-104	71087	TOU-A6-113	71097	TOU-A6-16	71107	TOU-A6-25	71117
TOU-A6-105	71088	TOU-A6-114	71098	TOU-A6-17	71108	TOU-A6-26	71118
TOU-A6-106	71089	TOU-A6-115	71099	TOU-A6-18	71109	TOU-A6-27	71119
TOU-A6-107	71090	TOU-A6-116	71100	TOU-A6-19	71110	TOU-A6-28	71120
TOU-A6-29	71121	TOU-A6-38	71131	TOU-A6-47	71141	TOU-A6-56	71151
TOU-A6-3	71122	TOU-A6-39	71132	TOU-A6-48	71142	TOU-A6-57	71152
TOU-A6-30	71123	TOU-A6-4	71133	TOU-A6-49	71143	TOU-A6-58	71153
TOU-A6-31	71124	TOU-A6-40	71134	TOU-A6-5	71144	TOU-A6-6	71154
TOU-A6-32	71125	TOU-A6-41	71135	TOU-A6-50	71145	TOU-A6-60	71155
TOU-A6-33	71126	TOU-A6-42	71136	TOU-A6-51	71146	TOU-A6-61	71156
TOU-A6-34	71127	TOU-A6-43	71137	TOU-A6-52	71147	TOU-A6-62	71157
TOU-A6-35	71128	TOU-A6-44	71138	TOU-A6-53	71148	TOU-A6-63	71158
TOU-A6-36	71129	TOU-A6-45	71139	TOU-A6-54	71149	TOU-A6-64	71159
TOU-A6-37	71130	TOU-A6-46	71140	TOU-A6-55	71150	TOU-A6-65	71160
TOU-A6-66	71161	TOU-A6-75	71171	TOU-A6-84	71181	TOU-A6-94	71191
TOU-A6-67	71162	TOU-A6-76	71172	TOU-A6-85	71182	TOU-A6-95	71192
TOU-A6-68	71163	TOU-A6-77	71173	TOU-A6-86	71183	TOU-A6-96	71193
TOU-A6-69	71164	TOU-A6-78	71174	TOU-A6-87	71184	TOU-A6-97	71194
TOU-A6-7	71165	TOU-A6-79	71175	TOU-A6-88	71185	TOU-A6-99	71195
TOU-A6-70	71166	TOU-A6-8	71176	TOU-A6-89	71186		
TOU-A6-71	71167	TOU-A6-80	71177	TOU-A6-9	71187		
TOU-A6-72	71168	TOU-A6-81	71178	TOU-A6-90	71188		
TOU-A6-73	71169	TOU-A6-82	71179	TOU-A6-91	71189		
TOU-A6-74	71170	TOU-A6-83	71180	TOU-A6-92	71190		

**Supplementary Table 2: Genetic architecture associated with the ‘super overyielding’ strategy with each of the two testers A1-137 and A6-55.** The list of the 22 QTLs detected across the three phenotypic traits, their physical position and their size are in columns 2-6. The identity of the 37 candidate genes underlying the 22 QTLs is given in columns 7 (ATG number) and 8 (locus name). The colored cells in column 8 illustrate the different categories of molecular functions (identified locus) of candidate genes, either related to transport/signaling functions (highlighted in light green), related to cell wall (highlighted in yellow), or corresponding to transcription factors (highlighted in dark green). Cells with a gradation of colors correspond to genes that fall into more than one category of molecular functions. The cells highlighted in purple indicate interesting candidate genes based on their identified functions, for validating their putative functioning with respect to super overyielding.

Chapter 2

Tester	QTL no.	chromosome	start	end	QTL size (kb)	ATG	Locus name
A1-137	1	1	1574123	1574693	0.57	AT1G05370	
						AT1G05380	
	2	1	12125340	12125344	0.00	AT1G33430	UPEX1
	3	1	20110977	20111074	0.10	AT1G53860	
						AT1G53870	
	4	1	20123542	20123639	0.10	AT1G53887	
						AT1G53890	
	5	1	23841746	23843975	2.23	AT1G64250	
						AT1G64255	
	6	1	26570101	26573969	3.87	AT1G70500	
						AT1G70505	
						AT1G70510	KNAT2
	7	1	26796601	26798438	1.84	AT1G71030	MYBL2
						AT1G71040	LPR2
	8	2	6467865	6467876	0.01	AT2G14980	
						AT2G14990	
	9 & 10	2	6683100	6683649	0.55	AT2G15340	
						AT2G15345	
	11	2	6947239	6947702	0.46	AT2G15950	
						AT2G15960	
						AT2G15970	COR34-PM1
	12	2	11643741	11643806	0.07	AT2G27220	BLH5
						AT2G27229	
	13	3	17719109	17720375	1.27	AT3G48000	ALDH2B4
						AT3G48010	CNGC16
	14	4	8441372	8441436	0.06	AT4G14723	CLL2
						AT4G14730	LFG1
	15	5	15541216	15541486	0.27	AT5G38810	
16	5	15552279	15554606	2.33	AT5G38840		
17 & 18	5	15769876	15771336	1.46	AT5G39410		
					AT5G39420	cdc2cAt	
A6-55	1	2	8193286	8193543	0.26	AT2G18910	
	2	2	9771103	9771140	0.04	AT2G22960	FPT2
	3	4	7725038	7725747	0.71	AT4G13260	YUC2
						AT4G13265	
4	5	26415793	26415825	0.03	AT5G66050		
					AT5G66052		

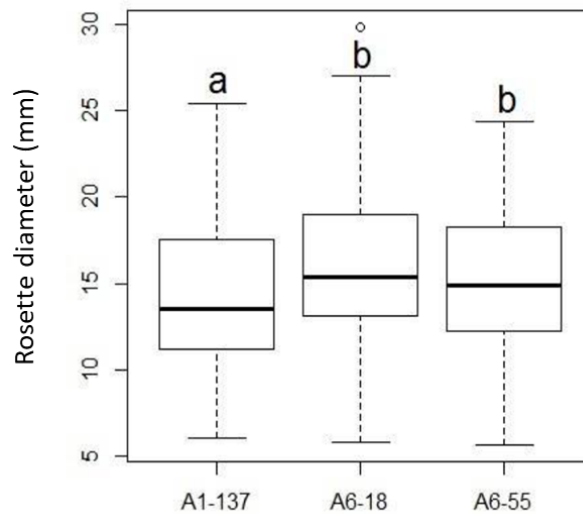
**Supplementary Figure 1: A triangular genomic space revealed by a Principal Component Analysis performed on the SNP matrix obtained for the 195 TOU-A accessions. Black and red dots correspond to the TOU-A1 and TOU-A6 accessions, respectively. The three tester accessions used in the experiment are highlighted in purple.**



**Supplementary Figure 2: Boxplots illustrating differences of rosette diameter of focal plants among the four treatments.** Different letters highlight significant differences obtained between the ‘solo’ treatment and the three intergenotype treatments based on Tukey’s test ( $P = 1 \times 10^{-9}$ ).



**Supplementary Figure 3: Boxplots illustrating differences of rosette diameter of tester plants among the three intergenotype treatments.** Different letters highlight significant differences obtained between the ‘A1-137’ treatment and the two other intergenotype treatments, based on Tukey’s test ( $P = 6 \times 10^{-4}$ ).



## *General discussion and perspectives*

*“in the long history of humankind (and animal kind, too) those who learned to collaborate and improvise most effectively have prevailed”*

*Charles Darwin, 1859*

## *I. Introduction*

Despite their importance in many of the key mechanisms involved in the functioning of both natural ecosystems and agro-ecosystems, plant-plant interaction studies still have two major gaps to be addressed, that is (i) the genetics of natural variation of plant-plant interactions and (ii) the relative importance of positive interactions within plant species. During my thesis, I decided to write two reviews to report the state-of-the-art related to these two major gaps. Firstly, the genetic and molecular mechanisms associated with natural variation of plant-plant interactions are not yet dissected thoroughly, even though the importance of identifying the genetic bases of plant-plant interactions has been highlighted for both crops and wild plant species (Subrahmaniam *et al.* 2018). For example, in crops, detection and characterization of QTLs underlying enhanced crop competitive ability, weed suppressive ability, or more recently overyielding can help in accelerating crop breeding programs. On the other hand, in wild plant species, understanding the genetic bases underlying plant-plant interactions is portrayed as essential for predicting the adaptive potential of natural plant communities (Pierik *et al.* 2013), especially to face current anthropogenic modifications of habitats (Frachon *et al.* 2019). In the first review, to explore the genetic architecture and the function of the candidate genes underlying natural plant-plant interactions (competition *versus* commensalism *versus* reciprocal helping *versus* asymmetry), we evaluated 63 quantitative trait loci (QTL) mapping and global gene expression studies based on plants directly challenged by other plants. These studies include 16 transcriptomic studies and 47 association mapping studies, all focused either on competitive interactions or on asymmetric interactions (mainly response to parasitic plants). The genetic architecture largely depends on the type of plant-plant interactions, ranging from a monogenic architecture for response to parasitic plants to the identification of medium effect and small effect QTLs for competitive interactions. This discrepancy of genetic architecture between the various plant-plant interacting systems nicely reflects the number of genes that have been cloned so far. While four genes conferring a resistance to parasitic plants have been cloned, no gene associated with natural variation of competitive interactions has been cloned to our knowledge. Furthermore, The candidate genes underlying natural plant-plant interactions could be classified into seven categories of plant function that have been identified in artificial environments either frequently (photosynthesis, hormones), only

## *General discussion and perspectives*

recently (cell wall modification and degradation, defense pathways against pathogens) or rarely (ABC transporters, histone modification and meristem identity/life history traits).

Secondly, natural plant communities can be ultimately disintegrated into populations assemblages of different species, wherein a population being composed of multiple genotypes. These local genotypes are bound to interact naturally in a social context. Intraspecific genotype-by-genotype (GxG) interactions within a population are an under-appreciated, but valuable aspect of community assembly since positive interactions are expected to be prominent at the local scale owing to natural neighbor interactions (Nowak 2006). In the second review, we identified 77 experiments providing evidence for intraspecific positive interactions. Both the kin selection theory and the elbow-room hypothesis were emphasized, despite their opposite predictions between the extent of genetic relatedness among neighbors and the level of positive interactions exhibited. Nonetheless, based on our survey, we identified three main gaps in the study of intraspecific plant –plant interactions, irrespective of the species studied. Firstly, to test for predictions about the underlying genomic architecture of positively interacting genotypes, *i.e.* testing both the kin selection theory and the elbow-room hypothesis, requires estimation of the degree of genetic relatedness among interacting genotypes. However, this crucial information has been poorly integrated into the study of positive GxG interactions at the intraspecific level. Secondly, positive interactions have been postulated to be selected under stressful environments at the interspecific level (Bertness & Callaway 1994). Theory suggests such cooperative links should exist among individuals from the same population because they all have shared co-evolutionary history experiencing similar range of both abiotic and biotic variables. Whether stress gradient hypothesis is also relevant at the intraspecific level remains an open question and demands an understanding of the identity of stresses that natural populations perceive in their natural habitats. In our survey, at most, only a rough description of habitats from which the genotypes have been collected was given in the studies for testing these hypotheses. Thirdly, at the intraspecific level, despite the demonstration of positive interactions in 27 plant species, the underlying genetic architecture and their adaptive status is still largely unknown. To our knowledge so far, only one study aimed to dissect the genetic bases of positive plant- plant interactions by mapping of a single QTL associated with natural variation of kin cooperation in a RIL family in *A. thaliana* (Wuest and Niklaus 2018).

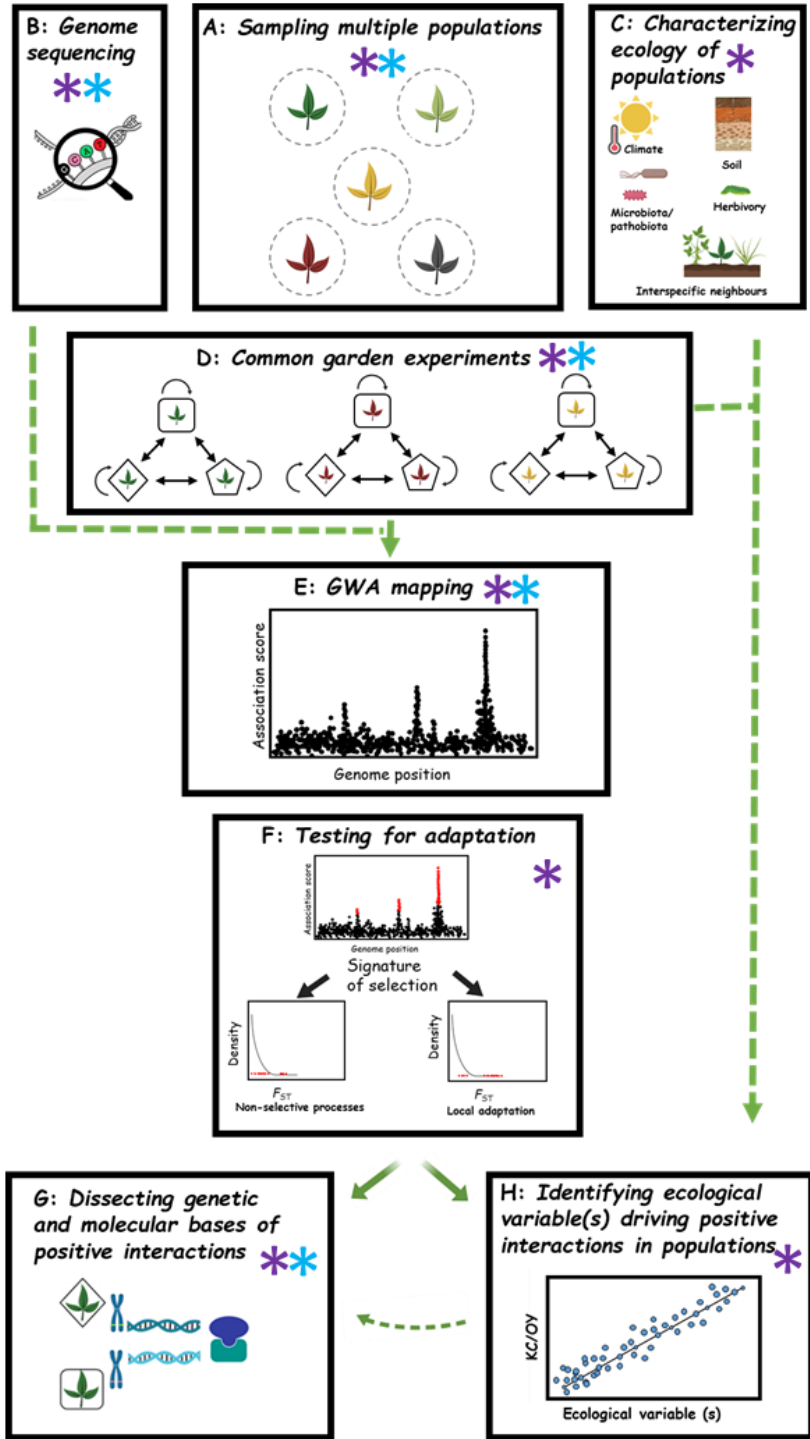
## General discussion and perspectives

The main objective of my thesis was therefore to understand the adaptive genetic bases of intraspecific positive plant-plant interactions in *A. thaliana*. To achieve this objective, I proposed in my second review a framework based on an interdisciplinary approach between quantitative genetics, ecology, genomics and association genetics (Figure 1). By following this framework, I studied the genetics of cooperation in *A. thaliana* at two complementary spatial scales, i.e. regional and local scales (Figure 1).

Therefore, the objectives of my first experimental chapter were to understand the genetics underlying natural variation of positive GxG interactions occurring at the *regional scale* and to identify the selective ecological factors driving variation of intraspecific positive interactions among wild populations of *A. thaliana*. Based on a field experiment to study natural variation of GxG interactions among 52 natural populations sampled at a regional scale, we were able to identify two different strategies of positive interactions, *i.e.* kin cooperation and overyielding. Interestingly, we identified fine scale biotic ecological factors putatively driving variation of positive interactions among these populations. Importantly, some of the genomic regions underlying this variation of positive interactions among the 52 populations carried signatures of local adaptation. Additionally, predominance of metabolism related gene functions underlying variation of positive interactions were explained for their putative roles in recruitment of similar microbiota by kins and potential complementary metabolite cross-feeding to explain overyielding.

At the *local scale*, in order to identify the presence of a ‘super overyielding’ strategy in *A. thaliana* and characterizing its underlying genetic architecture, a greenhouse experiment aimed at estimating the natural variation of intraspecific interactions within a local population is discussed in the second experimental chapter. Using genotypes collected from a population located in a highly diverse and competitive environment revealed the existence of certain genotypic combinations that were benefitting reciprocally to each other’s presence, resulting in a ‘super overyielding’ strategy. Genetic dissimilarity at the QTLs associated with this strategy was detected for such pairs. Therefore, one hypothesis to explain this cooperative behavior among super overyielders could be linked to direct/indirect cross-feeding among cooperative neighbours, similar to what has been observed for cooperation among bacterial strains.

General discussion and perspectives



**Figure 1: Framework for studying positive GxG interactions as proposed in Subrahmaniam *et al.* (submitted).** The asterisks in purple and in blue indicate steps followed in experimental chapter 1 and experimental chapter 2, respectively.

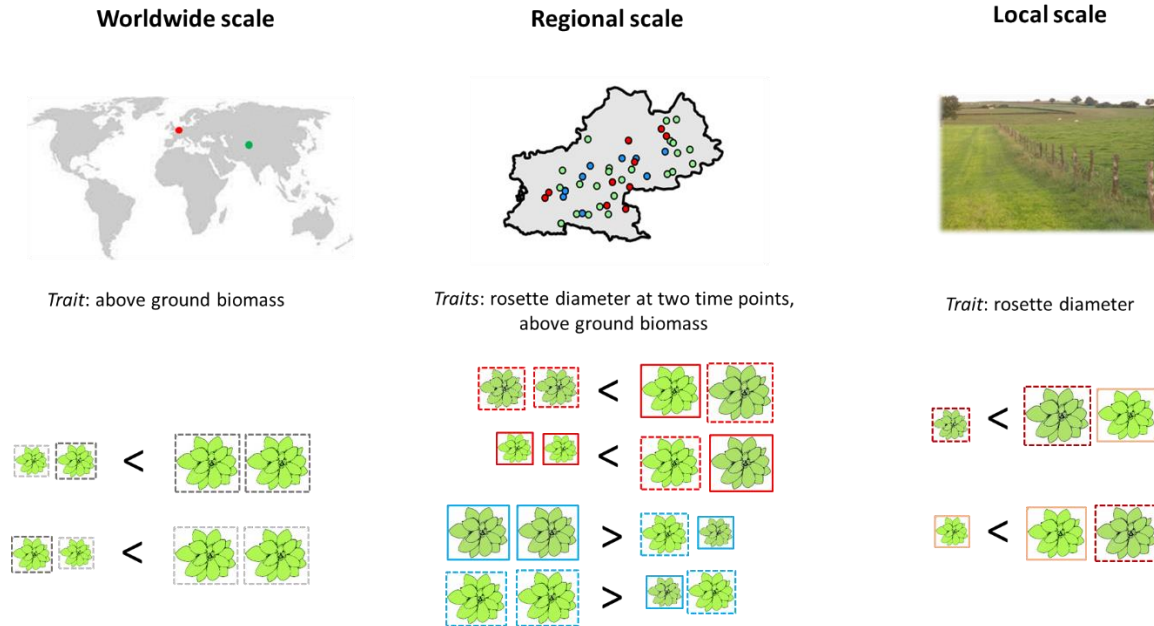
## *II. Natural genetic variation of intraspecific positive interactions at different geographic scales*

At the worldwide scale, kin cooperation was demonstrated recently in *A. thaliana* by using an experimental RIL population resulting from a cross between two worldwide accessions geographically separated by thousands of kilometers (Wuest and Niklaus 2018). However, as theory claims positive interactions to persist upon natural social interactions, use of accessions that have not co-existed in nature, may create an ecologically non-realistic setting for testing positive interactions. In a complementary way, I therefore decided to work at smaller geographical scales during my thesis. At a regional scale, I observed a large variation in the type of positive interactions among 52 natural populations from the Midi-Pyrénées, with the identification of both populations expressing a kin cooperation strategy and populations expressing an overyielding strategy. At a smaller geographical scale, I even observed a ‘super overyielding’ strategy, wherein each accession in some genotypic combinations were performing better than when growing alone. Interestingly, these ‘super overyielding’ combinations were highly dependent on the identity of the neighbouring genotype, suggesting presence of diffuse cooperative links with different genotypes within a population. Altogether, these results highlights the large diversity of positive interactions observed among and within natural populations of *A. thaliana* at contrasted geographical scales, thereby reinforcing the use of *A. thaliana* to establish genomic maps of local plant-plant cooperation.

However, one of the main drawbacks associated with these studies performed at all the three geographical scales is the small number of traits phenotyped. As has been demonstrated in the experimental chapter one, positive interactions may evolve during the life cycle of certain plant populations. Therefore, in order to obtain a full picture of the playful dynamics of traits involved in positive interactions, the need to measure multiple phenotypic traits representative of plant condition (i.e. traits related to leaves, roots, life history, seed production...) and/or a given phenotypic trait at multiple time points has been encouraged (Subrahmaniam *et al.* submitted). Future experiments in this direction should be based upon characterizing multiple traits for estimating plant-plant interactions in our natural populations of *A. thaliana*. Importantly, measuring belowground traits could be important to estimate potential tradeoff between

## General discussion and perspectives

aboveground and belowground resource partitioning upon neighbor interactions, which may be important indices in estimating different GXG interaction categories (from competition to cooperation).



**Figure 2: Illustration of experiments set up for identifying QTLs associated with natural variation of positive interactions in *A. thaliana* at different geographical scales.** The left column corresponds to the experiment conducted by Wuest & Niklaus (2018) at the worldwide scale, based on experimental RIL population collected from Netherlands (Bay-0 in red) and Tajikistan (Sha-0 in dark green). The experimental design for testing productivity in mono vs mixed culture based on RILs (indicated by different shades of gray) is depicted. The middle column depicts experiment conducted at the regional scale using 52 natural populations from the Midi-Pyrénées region. Experimental observations for both overyielding (genotypes in red color) and kin cooperation (genotypes in blue) are depicted. The right column correspond to the experiment conducted using the local population TOU-A where observations of ‘super overyielding’ genotypes are depicted.

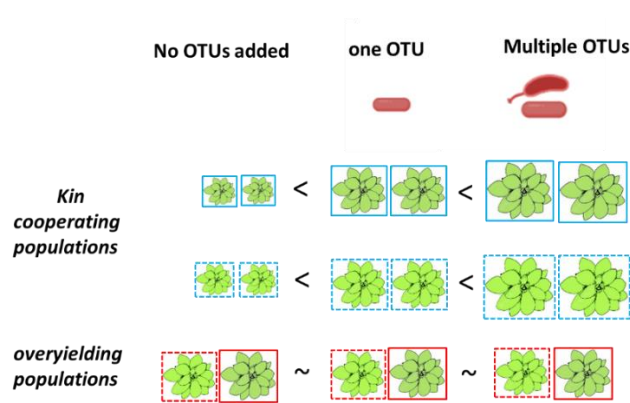
Another step will be to repeat the same experiments on other plant species to check the generality of the patterns of positive interactions among wild plant species. Within the team, the genetic material for starting these experiments are available for two other wild Brassicaceae species co-occurring with *A. thaliana* in natural plant communities in the Midi-Pyrénées regions, that is *Cardamine hirsuta* and *Erophila verna*.

### *III. Biotic factors as the main selective drivers of positive interactions?*

The fine-grained spatial variation in the degree of OY/KC observed in our study conducted at the regional scale was mainly linked to biotic factors. Our study, although based on a correlational approach, gave strong indications for the dependence of KC on the presence of specific commensal bacteria in native habitats of populations. On the other hand, we detected a strong and positive relationship between OY/KC variation and Shannon index of plant communities inhabited by *A. thaliana* in the Midi-Pyrénées region. These results throw some light into the prevalence of fine scale ecological variation driving positive interactions, thereby providing indirect indications towards existence of stress gradient hypothesis at the intraspecific level. They also provoke the urgent need for a better ecological characterization of natural habitats to identify the ecological factors driving positive interactions. Characterizing a large number of ecological factors for a substantial number of natural populations remains a tedious task. However, upon identifications of phenotype-ecology relationships, ecologically relevant stresses can be manipulated to test for their impact on the amount of positive interactions exhibited in some populations. For instance, the candidate ecological factors identified in our study could be tested for causal associations by designing experiments manipulating the said ecological variable. I illustrate the expectation of manipulating the number of candidate OTUs on the extent of kin cooperation in the populations found to exhibit kin cooperation in the experimental chapter one (Figure 3).

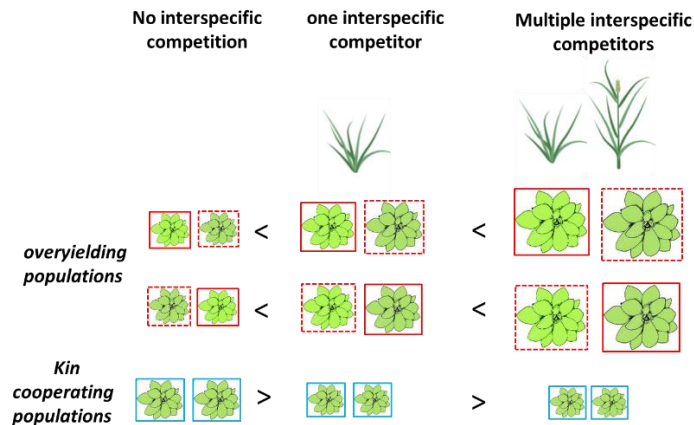
Based on observed correlations with the measurement of the first diameter measures, the cumulative presence of OTUs is suggested as a main driver of kin cooperation. One can hypothesize an increase in the extent of kin cooperation exhibited, based upon an increase in recruitment of the beneficial OTUs jointly by kins for reaping benefits of being associated together. Along with manipulating the presence of these OTUs, future experiments should also involve characterizing microbiota from the soil, rhizosphere, root and leaf compartments that may play important role in shaping plant neighbor interactions.

## General discussion and perspectives



**Figure 3: Illustration of the expectation on the role of commensal OTUs in driving kin cooperation in some local populations of the Midi-Pyrénées region.**

Based on observed correlations with biomass data, we also identified a correlation between the level of overyielding and Shannon index of plant communities. To test whether this relationship is causal, one can design similar experiments by manipulating the level of interspecific interactions (Figure 4). To check for variations in positive interactions exhibited, competitive interactions can be mimicked using plant species found associated with *A.thaliana* in the native habitats. Our results from the first experiment revealed correlation of positive interaction variation with abundance of one such species, *Cardamine hirsuta*. Hence, as a starting step, this species can be used to mirror competitive conditions in future experiments.



**Figure 4: Illustration of the expectation on the role of interspecific competition in driving overyielding in some local populations of the Midi-Pyrénées region.**

## *IV. What are the molecular determinants of cooperative plant-plant interactions?*

While hormonal regulatory pathways have been shown to be associated with the evolution of cooperation in many social insects and vertebrates (Supplementary Table 1 from Kasper *et al.* 2017), our results suggest the prevalence of plant metabolism- and development-related gene functions amongst the candidate genes that underlie variation of positive interactions at both regional and local scales. Regarding the putative involvement of metabolism related genes, a possible explanation could be complementary use of metabolic products by both partners, either directly or indirectly *via* differential microbiota recruitments. As a first step to check the validity of this hypothesis, we may consider to characterize the metabolite profiles of plants growing solo and in presence of different intraspecific neighbours.

Beside the metabolic component of these interactions, one important aspect to understand positive intraspecific interactions is to decipher the potential molecular dialogue between partners of the interaction. Indeed, in the second experiment, we identified some candidate genes related to signaling and regulatory pathways of plant developmental processes. These genes could have important functions for signaling the presence of a neighbor and for regulation of appropriate responses, notably for root and plant development potentially involved in cooperation dynamics.

Surprisingly, we did not identify in our experiments a preeminence of perception-associated functions, *i.e.* receptors, in contrast with previous results obtained on competitive interactions (Subrahmaniam *et al.* 2018). However, we cannot rule out the possibility that these mechanisms might exist and be essential for the establishment of cooperation. In order to identify such mechanisms, experiments can be conducted including barriers within the pot where intraspecific genotypes are grown together, thereby restricting root interaction and facilitating indirect interactions *via* exudates and/or associated microbiota. Such an experimental design should allow dissecting the role of each of these direct and indirect interaction mechanisms in positive interactions. For example, radioactive labelling of the metabolites produced by one genotype can be performed and then tracked in the cooperating neighbor. This should help to evaluate our hypothesis of complementary metabolite sharing among cooperating genotypes.

## General discussion and perspectives

RNA sequencing can also be envisaged for genotypes grown in solo, or in intragenotypic vs intergenotype treatments, to study changes in gene expression patterns, which can provide some cues about the different molecular pathways associated with the response to neighbor presence

Finally, cloning of the candidate genes identified in this thesis will also be carried out in the future. Since the experiments discussed here are based on utilizing natural accessions, use of the CRISPR/Cas9 system to create knockouts of the candidate regions followed by complementation with the natural alleles can be carried out for understanding the genetic and molecular mechanisms underlying variation of cooperative interactions between plants.

## V. References

- Bertness, M. D. & Callaway, R. (1994). Positive interactions in communities. *Trends in Ecology and Evolution*, 9(5), 187–191. [https://doi.org/10.1016/0169-5347\(94\)90087-6](https://doi.org/10.1016/0169-5347(94)90087-6)
- Frachon, L., Mayjonade, B., Bartoli, C., Hautekèete, N. C., & Roux, F. (2019). Adaptation to plant communities across the genome of *Arabidopsis thaliana*. *Molecular Biology and Evolution*, 36(7), 1442–1456. <https://doi.org/10.1093/molbev/msz078>
- Kasper, C., Vierbuchen, M., Ernst, U., Fischer, S., Radersma, R., Raulo, A., Cunha-Saraiva, F., Wu, M., Mobley, K.B., & Taborsky, B. (2017). Genetics and developmental biology of cooperation. *Molecular Ecology*, 26(17), 4364–4377. <https://doi.org/10.1111/mec.14208>
- Nowak, M. A. (2006). Five rules for the evolution of cooperation. *Science*, 314(5805), 1560–1563. <https://doi.org/10.1126/science.1133755>
- Pierik, R., Mommer, L., & Voesenek, L. A. (2013). Molecular mechanisms of plant competition: neighbour detection and response strategies. *Functional Ecology*, 27(4), 841–853. <https://doi.org/10.1111/1365-2435.12010>
- Subrahmaniam, H. J., Libourel, C., Journet, E. P., Morel, J. B., Muños, S., Niebel, A., Raffaele, S. & Roux, F. (2018). The genetics underlying natural variation of plant–plant interactions, a beloved but forgotten member of the family of biotic interactions. *The Plant Journal*, 93(4), 747–770
- Subrahmaniam, H. J., Roby, D. & Roux, F. (submitted to *Plant Communications*). Towards unifying evolutionary ecology and genomics to understand genotype-by-genotype interactions within wild plant species.
- Wuest, S. E., & Niklaus, P. A. (2018). A plant biodiversity effect resolved to a single chromosomal region. *Nature Ecology and Evolution*, 2(12), 1933–1939. <https://doi.org/10.1038/s41559-018-0708-y>

*VI. Supplementary information*

**Supplementary Table 1: Genetic and physiological mechanisms underlying cooperative behavior across taxa (from Kasper *et al.* 2017).** Examples of genetic and physiological mechanisms for cooperation across taxonomic groups indicating whether a genetic polymorphism (GP) has been identified, or whether differential gene expression in hormones and their receptors are responsible for phenotypic differences (DGE). We also present studies that identified differences in hormone levels (HL) or distribution of hormone receptors (RD) as the mechanism underlying cooperative phenotypes.

	System	Type of cooperative behaviour	Mechanism	GP	DGE	HL	RD	Citations
Proteo-bacteria	<i>Vibrio fischeri</i>	Quorum sensing/bioluminescence	<i>Ain</i> and <i>lux</i> proteins	X				(Lupp & Ruby 2005)
	<i>Mycococcus xanthus</i>	Fruiting bodies to survive starvation	PX mutation	X				(Fiegna <i>et al.</i> 2006)
Amoebozoa	Slime molds ( <i>Dictyostelium discoideum</i> )	Colony formation	<i>dimA</i>	X				(Foster <i>et al.</i> 2004)
	<i>Volvox carteri</i>	Reproductive altruism	<i>RegA</i> / <i>SAND</i> -like domain	X	X			(Kirk <i>et al.</i> 1999; Nedelcu & Michod 2006)
Fungi	Budding yeast ( <i>Saccharomyces cerevisiae</i> )	Biofilm-like cooperation	<i>FLO-1</i>	X				(Smukalla <i>et al.</i> 2008)
Animals	<i>Caenorhabditis elegans</i>	Social vs. solitary feeding behaviour	<i>Npr-1</i>			X	X	(Macosko <i>et al.</i> 2009)
	Honeybee ( <i>Apis mellifera</i> )	Division of reproductive castes and labour	Insulin-juvenile hormone-vitellogenin pathway, vitellogenin receptor, <i>Anarchy</i>		X	X		(Amdam <i>et al.</i> 2003; Corona <i>et al.</i> 2016; Ronai <i>et al.</i> 2016a; b)
	Termites ( <i>Cryptotermes secundus</i> )	Reproductive division of labour	<i>Neofem2</i>		X			(Korb <i>et al.</i> 2009)
	Ants ( <i>Solenopsis invicta</i> )	Monogynous/polygynous colony (supergene/‘social chromosome’)	<i>Gp-9</i>	X				(Keller & Ross 1998; Wang <i>et al.</i> 2013)
Insects	Ants ( <i>Polyrhachis moesta</i> )	Queens cooperate (have nest together)	Octopamine			X		(Koyama <i>et al.</i> 2015)
	Earwigs ( <i>Forficula auricularia</i> )	Parent-offspring co-adaptation, maternal care	PebIII, Th, juvenile hormone-vitellogenin pathway and vitellogenin receptor		X			Wu <i>et al.</i> , unpublished
	Burying beetles ( <i>Nicrophorus vespilloides</i> )	Parental care	juvenile hormone-vitellogenin pathway and vitellogenin receptor		X			(Roy-Zokan <i>et al.</i> 2015; Engel <i>et al.</i> 2016)
	Gold fish ( <i>Carrasius auratus</i> )	Social approach behaviour, vasotocine inhibits social approach, isotocine stimulates it	arginine vasotocin/isotocin			X		(Thompson & Walton 2004)
Teleosts	Lamprologine cichlids	Social behaviour expression	arginine vasotocin/arginine vasotocin receptor		X			(O’Connor <i>et al.</i> 2015; Reddon <i>et al.</i> 2015)
	Ruff ( <i>Philomachus pugnax</i> )	Cooperative male-male relationships (different mating phenotypes) in leks	Androsteroid homeostasis (HSD17B2)	X				(Küpper <i>et al.</i> 2015; Lamichhane <i>et al.</i> 2015)
Birds	Great tits and blue tits ( <i>Parus major</i> , <i>Cyanistes caeruleus</i> )	Parental care and corticosterone levels covary	corticosterone, testosterone			X		studies cited in (Hau & Goymann 2015)
	White-throated sparrows ( <i>Zonotrichia albicollis</i> )	Parental care; monogamy; aggression differences between white and tan morph (morph is det. by supergene)	Steroid metabolism; serotonin	X				(Tuttle <i>et al.</i> 2016)

Supplementary Table 1 (continued)

Mammals	Meerkats ( <i>Suricata suricatta</i> )	'Cooperative syndrome' (increased digging, guarding, pup-feeding and associating with pups; decreased aggression)	oxytocin				X	(Madden & Clutton-Brock 2011)
	Domestic dog ( <i>Canis lupus</i> )	Social bonds	oxytocin				X	(Romero <i>et al.</i> 2014)
	Rodents general	Species differences in alloparental care	oxytocin receptor					(Olazábal & Young 2006)
	Mice ( <i>Mus musculus</i> )	Communal nesting & lactation	oxytocin				X	(Harrison <i>et al.</i> 2016)
	Rats ( <i>Rattus norvegicus</i> )	Maternal care; epigenetic (cytosine methylation)	Estrogen receptor ER- $\alpha$		X			(Champagne <i>et al.</i> 2006)
	Rats ( <i>Rattus norvegicus</i> )	coordination during cooperative task	Norepinephrine, serotonin, dopamine				X	(Tsoury <i>et al.</i> 2012)
	<i>Peromyscus californicus</i> , <i>P. maniculatus</i> (monogamous vs polygamous species)	Pair bond as a proxy for group living	ViaR/ oxytocin receptor				X	(Young <i>et al.</i> 2001; Hammock & Young 2005; Anacker & Beery 2013)
	Prairie voles ( <i>Microtus ochrogaster</i> )	Reduced alloparental effort in males when treated with antagonist	oxytocin				X	(Bales & Carter 2003)
	Tuco-tucos ( <i>Ctenomys sociabilis</i> & <i>C. haigi</i> )	Communal nesting	oxytocin receptor				X	(Anacker & Beery 2013)
	Naked mole rats ( <i>Heterocephalus glaber</i> )	Cooperative breeding/ eusociality	oxytocin receptor				X	(Anacker & Beery 2013)
	New World primates general	Social behaviour	oxytocin / oxytocin receptor and arginine vasopressin /V1a receptor		X			(Bergey <i>et al.</i> 2016; French 2016)
	Vervet monkeys ( <i>Chlorocebus aethiops</i> , <i>C. pygerythrus</i> )	Parental care/parent-offspring interaction	dopamine receptor D4		X			(Kaiz <i>et al.</i> 2010; Fairbanks <i>et al.</i> 2012)
	Chimpanzees ( <i>Pan troglodytes</i> )	Building cooperative relationships; oxytocin levels elevated after grooming	oxytocin				X	(Crockford <i>et al.</i> 2013)
	Chimpanzees ( <i>Pan troglodytes</i> )	Kin and non-kin bonds, food sharing	oxytocin				X	(Wittig <i>et al.</i> 2014)
	Humans ( <i>Homo sapiens</i> )	Pro-social behaviour (dictator game)	arginine vasopressin receptor 1a; receptor S 3 repeat		X			(Avinun <i>et al.</i> 2011; Knaf <i>et al.</i> 2008)
		Pro-social behaviour (dictator game; social value orientation task), Autism	oxytocin receptor		X			(Israel <i>et al.</i> 2009; Di Napoli <i>et al.</i> 2014)
		Social behaviour/ parental care/parent-offspring interaction. Self-reported social behaviour	dopamine receptor D4, dopamine receptor D5, IGF2		X			(Bakermans-Kranenburg & Ijzendoorn 2006; Sheese <i>et al.</i> 2007)
		Donation behaviour	COMT Val158Met (rs4680)		X			(Reuter <i>et al.</i> 2011), but see (Anacker & Beery 2013) (no effect)
		Self-reported social behaviour	GABRB2 (rs187269)		X			(Tsang <i>et al.</i> 2013)

## Abstract

Despite the importance of plant-plant interactions in the functioning of both natural ecosystems and agro-ecosystems, studies on plant-plant interactions still have two major gaps to be addressed, that is (i) the genetics of natural variation of plant-plant interactions and (ii) the relative importance of positive interactions within plant species. After writing two reviews on the state-of-the-art related to these gaps, I decided to understand the adaptive genetic bases of intraspecific positive plant-plant interactions in *Arabidopsis thaliana* at two geographical scales. To do so, I adopted an interdisciplinary approach between quantitative genetics, ecology and genome-wide association mapping. Firstly, based on a field experiment designed to study natural variation of genotype-by-genotype interactions among 52 whole-genome sequenced natural populations from the Midi-Pyrénées region, I identified two different strategies of positive interactions, *i.e.* kin cooperation and overyielding. Natural variation of positive interactions among these populations were mainly associated with biotic ecological factors varying at a fine spatial scale, such as presence of commensal bacteria or Shannon index of plant communities in the native habitats. Importantly, QTLs associated with variation of positive interactions were significantly enriched in genomic signatures of local adaptation. Secondly, based on a greenhouse experiment using 195 whole-genome sequenced accessions collected in a local French population located in a highly diverse and competitive environment, we revealed the existence of certain genotypic combinations that were benefitting reciprocally to each other's presence, resulting in a 'super overyielding' strategy. Importantly, genetic dissimilarity at the QTLs associated with this strategy was detected for such pairs, supporting the 'compatibility genes' hypothesis as underlying this 'super overyielding' strategy. Finally, at both geographical scales, we detected a predominance of metabolism related gene functions underlying natural variation of positive interactions, which might be explained by their putative roles in (i) recruitment of similar microbiota by kin to explain kin cooperation, and (ii) potential complementary metabolite cross-feeding to explain overyielding. The next step is undoubtedly cloning of the candidate genes to identify causal associations, thereby allowing to start getting a glimpse on the genetic and molecular landscape associated with positive interactions in *A. thaliana*.

**Keywords:** intraspecific variation, genotype-by-genotype interactions, cooperation with reciprocal benefit, kin cooperation, overyielding, local adaptation, ecological selective agents, association genetics.

## Résumé

Malgré l'importance des interactions plante-plante dans le fonctionnement des écosystèmes naturels et des agro-écosystèmes, les études sur les interactions plante-plante ont encore deux lacunes importantes à combler, à savoir (i) la génétique associée à la variation naturelle des interactions plante-plante et (ii) l'importance relative des interactions positives au sein des espèces végétales. Après avoir rédigé deux revues faisant un état de l'art sur chacune de ces lacunes, j'ai décidé de m'intéresser à l'étude des bases génétiques adaptatives des interactions plante-plante positives chez *Arabidopsis thaliana* à deux échelles géographiques. Pour cela, j'ai adopté une approche interdisciplinaire entre génétique quantitative, écologie et génétique d'association pangénomique. Dans un premier temps, sur la base d'une expérience réalisée sur un terrain expérimental conçue pour étudier la variation naturelle des interactions génotype-génotype entre 52 populations naturelles de la région Midi-Pyrénées, j'ai identifié deux stratégies contrastées d'interactions positives, à savoir la coopération entre apparentés (kin cooperation) et la surproduction (overyielding). La variation naturelle des interactions positives entre ces populations était principalement associée à des facteurs écologiques biotiques variant à une fine échelle spatiale, tels que la présence de bactéries commensales ou l'indice de Shannon des communautés végétales dans les habitats natifs. Par ailleurs, les QTL associés à la variation des interactions positives sont significativement enrichis en signatures génomiques d'adaptation locale. Dans un deuxième temps, à partir d'une expérience en serre basée sur 195 accessions collectées dans une population locale française située dans une communauté végétale très diversifiée et compétitive, nous avons révélé l'existence de certaines combinaisons génotypiques où chaque accession bénéficie réciproquement de la présence de l'autre accession, résultant en une stratégie de 'super overyielding'. De manière intéressante, nous avons trouvé que les accessions coopératives étaient génétiquement très différenciées au niveau des QTL associés à cette stratégie, ce qui soutient l'hypothèse des 'gènes de compatibilité' comme étant à la base de cette stratégie de 'super overyielding'. Finalement, aux deux échelles géographiques, nous avons identifié que la variation naturelle des interactions positives étaient associées à une prédominance de fonctions génétiques liées au métabolisme, ce qui pourrait s'expliquer par leurs rôles potentiels dans (i) le recrutement de microbiotes similaires par deux plantes ayant le même génotype pour expliquer la coopération entre apparentés, et (ii) l'alimentation croisée complémentaire de métabolites pour expliquer la surproduction. La prochaine étape est sans aucun doute le clonage des gènes candidats pour identifier les associations causales, ce qui pourrait permettre de commencer à avoir un aperçu du paysage génétique et moléculaire associé aux interactions positives chez *A. thaliana*.

**Mots clés:** variation intraspécifique, interactions génotype-génotype, coopération avec bénéfiques réciproques, kin cooperation, overyielding, adaptation locale, agents écologiques sélectifs, génétique d'association.