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1 **Kin selection, kin recognition and kin discrimination in plants revisited: a**  
2 **claim for considering environmental and genetic variability**

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19 **Keywords:** plant-plant interactions, cooperative behaviour, long-lived species, intraspecific  
20 variation, context-dependency.

21 **Abstract**

22 Considering kin selection in the study of interacting conspecific plants broadens our vision of  
23 plant behaviour and brings arguments to explain plant-plant positive interactions. These  
24 interactions are the subject of abundant research in community ecology and the role of  
25 relatedness in interactions between individuals has become a hot topic. Indeed, the past decade  
26 has seen a steady accumulation of exciting but also controversial results regarding the behaviour  
27 of plants in the presence of genetically related neighbours, i.e., kin discrimination. In addition  
28 to the existence of some methodological and semantic aspects that should be strengthened to  
29 accurately reveal the occurrence of kin recognition or selection, we claim here that some key  
30 aspects must be considered to increase our ability to detect kin recognition and selection in  
31 plants. These aspects include intraspecific variability, the fact that plant-plant interactions are  
32 context-dependent, and the lifespan of species (particularly long-lived ones). Additionally, we  
33 note that the study of the population spatial genetic structure (SGS) could be a useful tool to  
34 identify candidate settings for the study of kin recognition and selection in plants.

35

## 36 **Introduction**

37 Plants are sessile organisms and cannot chose or move away from their neighbours, but are tied  
38 to them throughout their life. In this context, identifying the factors that can influence the type  
39 of interactions (positive, neutral or negative) between individuals is of prime importance. One  
40 such factor that has become increasingly popular is genetic relatedness between individuals.  
41 Recent research has shown compelling evidence that plants are able to detect their close  
42 neighbours and actively interact with them. Indeed, plants exhibit complex recognition and  
43 communication mechanisms (Bilas, Bretman & Bennett 2021). Classically studied between  
44 different plant species, these interactions also apply at the intraspecific level (Bilas, Bretman &  
45 Bennett 2021). Remarkably, plants can distinguish their conspecific neighbours based on their  
46 genetic relatedness (i.e., kin recognition) and adjust their 'behaviour' accordingly (kin  
47 discrimination) (Ehler & Bilde 2019, Anten & Chen, 2021a). Being able to identify related  
48 individuals could be advantageous to reduce competition among relatives (File, Murphy &  
49 Dudley 2012), a cooperative behaviour that can be promoted through kin selection. However,  
50 traits that reduce competition can be costly for the individual (e.g., limiting resource acquisition;  
51 Dudley, Murphy & File, 2013). Kin selection theory (Hamilton 1964) states that an altruistic  
52 trait can be favoured by natural selection when the altruist and the recipient are related. This is  
53 based on the concept of indirect fitness, i.e., indirect benefits accrued by the altruistic individual  
54 through the fitness of the recipient, as the two individuals share genes. If individuals are  
55 sufficiently closely related, altruism can favour the reproduction of the set of genes they share  
56 despite losses in direct fitness by the altruist individual (Axelrod & Hamilton 1981). Note that  
57 kin recognition and kin discrimination are not necessary for kin selection to occur (e.g., in  
58 viscous populations, cooperation with neighbours is mostly directed towards kin; West et al.,  
59 2007).

60 In a recent review, Anten & Chen (2021a) provide a critical and detailed overview of the  
61 conceptual bases and latest results regarding kin recognition, kin discrimination and kin  
62 selection in plants. In summary, they state that most of the studies have so far explored plant-  
63 plant interactions at the root level. A frequently observed result is that plants growing with  
64 related individuals show a reduction in belowground biomass compared to plants growing with  
65 strangers (usually seeds from different mothers), which is interpreted as a reduction in  
66 competition towards relatives, possibly leading to positive interactions (File, Murphy & Dudley  
67 2012). The possible mechanisms of belowground kin recognition are root-exudates and  
68 mycorrhizal networks. Although some methodological concerns are discussed by Anten &

69 Chen (2021a), the authors conclude that there is sufficient evidence for supporting kin  
70 recognition in plants.

71 Anten & Chen (2021a) accomplished the best update so far regarding kin discrimination  
72 and kin selection in plants. We think, nevertheless, that some aspects were not considered in  
73 their review that are of great complementary importance to broaden the kin recognition and  
74 selection framework: intraspecific variability, environmental context dependency, plant  
75 longevity and spatial patterns of plant interactions. We expand on these underexplored aspects,  
76 which we consider essential to build a testable framework about kin discrimination and kin  
77 selection and expose two case studies that exemplify these new aspects. We then develop two  
78 additional points:(i) several papers confuse (or use alternatively) the concepts of kin  
79 recognition, kin discrimination and kin selection for which we offer a clarification; (ii) this  
80 confusion often leads to methodological issues and we propose methodological guidelines for  
81 experimental designs to detect kin discrimination and assess kin selection.

82

### 83 **Should we all cooperate? The neglected role of genetic diversity for kin interaction** 84 **mechanisms**

85 Several published studies, including Anten & Chen's (2021a) review, have concluded that kin  
86 recognition is either present or absent in a given species. However, do we expect species to be  
87 entirely homogeneous with regard to kin discrimination or even kin selection? In other words,  
88 is it reasonable to assume that all individuals of the same species exhibit the same 'behaviour'?  
89 Probably not, as already suggested by several authors (Clark 2010; Bolnick et al., 2011;  
90 Westerband, Funk & Barton 2021). For example, it is recognised that intraspecific diversity in  
91 plants shapes biotic and abiotic interactions and plays a key role in determining the performance  
92 of individuals (Westerband, Funk & Barton 2021). Intraspecific diversity is the rule for a wide  
93 range of traits (Siefert et al., 2015), it is therefore conceivable that not all individuals within a  
94 species possess the same traits associated with kin recognition, and that not all individuals have  
95 the (same) ability to distinguish their related neighbours. Moreover, our understanding of the  
96 genetic and molecular bases underlying the natural variation of plant-plant interactions remains  
97 limited and needs to be considered in further research (Subrahmaniam et al., 2018). We assert  
98 here that intraspecific variability for kin recognition has been poorly addressed thus far,  
99 although it represents a critical issue.

100 Theoretical models exploring kin recognition in various organisms (mainly on animals,  
101 but the results are valid for all species capable of kin recognition) predict a wide range of  
102 intraspecific variation in kin recognition mechanisms between and within populations (see  
103 review by Penn & Frommen 2010), although other models do not predict kin recognition  
104 mechanisms at equilibrium because these mechanisms are costly (Rousset & Roze 2007). We  
105 argue that kin recognition in plants could still be an advantageous local strategy in certain  
106 conditions while not being advantageous when the population reaches equilibrium. Thus,  
107 intraspecific diversity for kin interaction mechanisms should be particularly important to  
108 consider. Anten & Chen (2021a) and also Fréville et al. (2021) discussed this point in the case  
109 of crops, which exhibit lower genetic diversity than wild species and where individuals interact  
110 mostly with kin and proposed that studies include sampling replication at the genotypic level to  
111 capture sufficient intraspecific variation in kin discrimination. Most studies have used only a  
112 few different kin groups (usually two to four) and thus captured only a small part of the existing  
113 variability in the species. Yet, some recent studies have made the effort to include a larger  
114 number of kin groups (eight-ten and up to 28 kin groups) (Semchenko, Saar & Lepik 2017;  
115 Torices, Gómez & Pannell 2018; Takigahira & Yamawo 2019). To overcome this important  
116 issue, which can lead to erroneous conclusions at the species level, we advocate the use of at  
117 least five to ten kin groups originating from several populations. The logic is as follows: the  
118 probability of detection depends on the frequency of the trait. If one in five individuals in a  
119 population can discriminate kin, using five kin groups there is still a 33% chance of missing the  
120 trait (i.e., reaching erroneous conclusions), and this value is reduced to 11% with ten kin groups.  
121 If the frequency is one in ten, the chances are 60% and 35%, respectively.

122

### 123 **Under which conditions is it beneficial to cooperate with relatives?**

124 Because plants are sessile organisms, they cannot move to avoid their neighbours' effects nor  
125 environmental stress. Local adaptation (i.e., selection for traits that increase the fitness of  
126 individuals in their local environment) and phenotypic plasticity (i.e., the ability of a given  
127 genotype to express different phenotypes according to some environmental cues or  
128 circumstances) are the two common processes to overcome these threats (e.g., de Villemereuil,  
129 Mouterde, Gaggiotti & Till-Bottraud 2018; Fox, Donelson, Schunter, Ravasi & Gaitán-Espitia  
130 2019). Phenotypic variability can thus be induced by variation in the local conditions and could  
131 influence the nature of plant-plant interactions (Callaway, Pennings & Richards 2003). As a  
132 consequence, should we expect the same type of interactions between individuals living under

133 different conditions? The stress gradient hypothesis (SGH, Bertness & Callaway 1994) predicts  
134 that the level of stress will influence the type of interaction between individuals shifting from  
135 positive (i.e., facilitation) in stressful environments to negative (i.e., competition) in favourable  
136 environmental conditions (Bertness & Callaway 1994). Facilitation is defined as ‘an interaction  
137 in which the presence of one species alters the environment in a way that enhances the growth,  
138 survival and reproduction of a second species’ (Bronstein 2009, p.1160). Interactions show  
139 ‘conditionality’ when costs and benefits, and thus outcomes, are affected in predictable ways  
140 by ecological conditions (Bronstein 1994). Plant interactions thus depend on the ecological  
141 settings in which they take place, and in particular, plants adjust to the level of stress (Bilas,  
142 Bretman & Bennett 2021). It has been implicitly assumed that facilitation only occurs between  
143 individuals of different species, but as shown and discussed by Fajardo & McIntire (2011),  
144 facilitation can be found between individuals of the same species and was indeed suggested in  
145 several other cases (Proença et al., 2019; Reijers, Akker, Cruijsen, Lamers & Heide 2019).  
146 Facilitation allows, or even favours, the coexistence of individuals of the same species in close  
147 proximity, i.e., within their zone of interaction (ZOI *sensu* Stoll & Weiner 2000). If the ZOI of  
148 related individuals overlaps this would allow kin recognition, kin discrimination and kin  
149 selection mechanisms to operate. Following SGH predictions, harsh environmental conditions  
150 should lead to stronger positive interactions (i.e., cooperation) if related individuals are present.  
151 We can thus expect contrasting responses for individuals living in stressful versus benign  
152 environments and thus, in a changing environment, the same closely related individuals could  
153 cooperate or compete depending on the level of stress.

154           So far, few studies have shown evidence that kin recognition depends on the level or the  
155 type of environmental stress. In these studies, kin recognition and discrimination responses  
156 were triggered by several abiotic factors including nutrient availability, for example, in *Glycine*  
157 *max*, *Caesalpinia pulcherrima*, *Populus tomentosa* (Li, Xu & Liu 2018), and *Pisum sativum*  
158 (Pezzola, Pandolfi & Mancuso 2020), heavy metal concentrations in *Sorghum vulgare* (Li, Xu  
159 & Feng, 2018) and biotic factors such as the presence of another species in *Medicago* genera  
160 (Ehlers, David, Damgaard, & Lenormand 2016). On the other hand, water availability, did not  
161 trigger kin recognition responses in *Glechoma hederacea* (Goddard, Varga, John & Soulsbury  
162 2020). Thus, it is possible that kin recognition is only expressed in a given kin group under  
163 certain ecological conditions, which can only be identified by modulating the type of stress and  
164 its intensity. We note then that it is essential to include both several stressors and several

165 genotypes (kin groups) in any experimental effort to elucidate kin recognition/selection in  
166 plants.

167 Kin discrimination also depends on whether cooperation is obligate or facultative. In the  
168 case of obligate cooperation, kin recognition is ultimately not useful as individuals always  
169 cooperate whatever the identity of their neighbour is and there is no discrimination. In contrast,  
170 facultative cooperation implies that individuals exhibit phenotypic plasticity for cooperation  
171 and that they are able to distinguish relatives (i.e., present kin recognition). Both phenotypic  
172 plasticity (Ghalambor et al., 2007) and kin recognition (Rousset & Roze 2007) have associated  
173 costs. To identify cases where obligate or facultative cooperation is favoured requires thus to  
174 estimate the costs and benefits of facultative cooperation (plasticity) in addition to those of  
175 Hamilton's equation (Hamilton 1964), weighted by the probability of interacting with a relative.  
176 Therefore, in the case of facultative cooperation, the question is not only with whom but also  
177 under what conditions should individuals cooperate? In a stable environment, the phenotype is  
178 constant and obligate cooperators would be favoured. In a changing environment, SGH suggests  
179 that interactions shift between positive and negative and being a facultative cooperator would  
180 be advantageous. In the latter case, cooperation will depend on the relatedness between  
181 individuals. The important point here is that environmental conditions in which the experiment  
182 takes place, but which are seldom considered in kin studies, condition the detection of kin  
183 recognition/selection.

184

### 185 **Cooperating throughout life? Interactions in long-lived species**

186 For practical reasons, most of the studies on kin recognition thus far have focused on annual or  
187 short-lived plants (Subrahmaniam, Roby & Roux 2021). Few studies have investigated long-  
188 lived species such as perennial herbs, shrubs or even trees (Till-Bottraud, Fajardo & Rioux  
189 2012; Fajardo, Torres-Díaz & Till-Bottraud 2016; Pickles et al., 2017), and mostly addressed a  
190 short period of their lives (e.g., the seedling stage; Takigahira & Yamawo 2019; He et al., 2021).  
191 Some authors reported positive interactions between mature related individuals following  
192 herbivory, through discrimination of volatile chemicals emitted from relatives (Karban et al.,  
193 2014; Hussain, Rodriguez-Ramos & Erbilgin 2019). Other showed that related seedlings grow  
194 better in the presence of kin (Assay et al., 2020) and benefit more from the mycorrhizal network  
195 shared with related neighbours (Pickles et al. 2017).



196           However, long-lived species exhibit several interesting characteristics that have not  
197 been much considered: (i) the long lifespan means that an individual will likely experience  
198 different environmental situations during its life; (ii) different life stages may have very  
199 different ecologies (ontogenetic stages, e.g., seedling *vs.* mature tree, related to the regeneration  
200 niche, Grubb 1977), kin discrimination and kin selection might thus be advantageous at a given  
201 developmental stage but not at others. For example, at early stages when seedling mortality is  
202 important because they are more sensitive to harsh conditions, positive interactions between  
203 related individuals would ensure better survival of related genotypes; (iii) some long-lived  
204 species, particularly trees, strongly affect their physical environment by improving their living  
205 conditions (i.e., the ecosystem engineering effect *sensu* Jones, Lawton & Shachak 1994),  
206 modulating the level of stress and improving resource availability through their effects on the  
207 local environment (McIntire & Fajardo 2014). As this effect is incremental through time it will  
208 be all the more important in long-lived species (Hortobágyi, Corenblit, Steiger & Peiry 2018).  
209 As a given individual can experience different conditions through its lifetime, the chance of  
210 detecting kin recognition could strongly depend on the life stage considered. This particular  
211 point is essential to consider when conducting field studies where local conditions can change  
212 or even be improved by engineering species. All these three characteristics are linked to the  
213 SGH, although the gradient here is temporal as long-lived species interact with their neighbours  
214 through decades or even centuries in a fluctuating or changing micro- and macro-environment.

215           Moreover, in seasonal environments, the physiological activity is reduced or potentially  
216 halted by dormancy periods. Is this interaction between two long-lived individuals just one  
217 absolute interaction or is it repeated interactions (e.g., in the different growing seasons), which  
218 would lead to radically different outcomes (Axelrod & Hamilton 1981)? This is still an open  
219 question, for which evolutionary game theory (Maynard Smith 1982) could provide a  
220 theoretical framework.

221

## 222 **Spatial patterns of plant interactions: the added value of spatial genetic structure**

223           One potentially valuable tool to search for positive interactions among kin in natural  
224 populations is the spatial genetic structure (SGS). The SGS, defined as the non-random spatial  
225 distribution of genotypes, characterizes the relationship of relatedness estimates between pairs  
226 of individuals and their physical proximity (Loiselle, Sork, Nason & Graham 1995; Hardy  
227 2003). The genetic composition of a population has an influence on the type of biotic interaction

228 that can occur among immediate neighbours (Fajardo, Torres-Díaz & Till-Bottraud 2016).  
229 Using a spatial genetic approach can thus offer complementary insights, particularly for *in situ*  
230 studies. A significant SGS indicates that related individuals coexist close to each other, and  
231 thus, the possibility of kin interactions increases. This makes SGS an interesting tool to detect  
232 the presence of related individuals and to consider potential positive interactions in the context  
233 of kin recognition and selection. Moreover, this SGS approach seems particularly relevant to  
234 us as kin selection can evolve in viscous populations without the need for kin recognition or  
235 discrimination (e.g., obligate cooperation) (Waldman 1988, Gardner & West, 2004). However,  
236 the fact that individuals are “close to each other” does not mean that they will necessarily  
237 interact. Plant–plant interactions are inherently local in nature. They mostly occur among  
238 immediate neighbours, in which case an individual’s direct effect on another (negative or  
239 positive) will have a delimited influence in space, i.e., the ZOI (Stoll & Weiner 2000). The ZOI  
240 will grow as individuals grow because individuals, in particular trees, will occupy a larger area  
241 with their aerial and belowground organs. Thus, over their lifetime, individuals will interact  
242 with other individuals located further away as their ZOI expands, and thus allowing more or  
243 different individuals to interact through time. Biotic interactions will be influenced by the local  
244 SGS (i.e., the presence of related individuals within the ZOI) but can also significantly influence  
245 the SGS within the ZOI of individuals through the survival of related individuals in local  
246 patches, which can be monitored through time. However, very few studies have linked the SGS  
247 and the ZOI when interactions between related individuals take place (Till-Bottraud, Fajardo &  
248 Rioux 2012; Segovia, Vásquez, Faugeron & Haye 2015; Fajardo, Torres-Díaz & Till-Bottraud  
249 2016).

250

### 251 **The relationship between the SGS and intraspecific interactions: two case studies in long-** 252 **lived species**

253 To illustrate how all the aspects we have outlined above could help us assess the ultimate  
254 importance of kin selection, we describe here two examples of positive interactions between  
255 related individuals in tree species.

256 First, in *Nothofagus pumilio* (Nothofagaceae) second-growth stands located in the  
257 Chilean Patagonia, multi-stemmed trees are formed by related individuals. They are restricted  
258 to forest edges where stress (wind and desiccation) was very high at the regeneration stage, and

259 where facilitation increased seedling survival (Fajardo & McIntire 2011) and growth in multi-  
260 stemmed adult trees (Fajardo & McIntire 2010; McIntire & Fajardo 2011). Trees from different  
261 multi-stemmed clusters are unrelated, and no group dispersal mechanism is reported for this  
262 species (Till-Bottraud, Fajardo & Rioux 2012; Fajardo, Torres-Díaz & Till-Bottraud 2016)  
263 indicating better survival when related seedlings happen to grow together. The ecological  
264 mechanism involved here may be an altruistic or cooperative fusion of trunks, which would be  
265 physiologically and biomechanically selectively advantageous for resource acquisition or  
266 stability against wind (Tarroux & DesRochers 2011; Tarroux, DesRochers & Tremblay 2014;  
267 Fajardo, Torres-Díaz & Till-Bottraud 2016), but fusion may also involve some costs (e.g.,  
268 resource sharing, pathogen transmission). Stem merging scales up the level of organization to  
269 the group and therefore competition does not occur among individuals but among groups of  
270 trees (McIntire & Fajardo, 2011). In a kin selection framework, fusions would be selected and  
271 would only occur between related individuals, as indirect fitness gains might offset these costs.

272 The other example is the Black Poplar (*Populus nigra* L. Salicaceae) in a riparian  
273 context. At the establishment phase, *P. nigra* seedlings grow in high densities on exposed  
274 alluvial bars of rivers, and facilitation is the major occurring interaction (Corenblit et al. 2018).  
275 Young individuals form dense patches and mechanically protect each other from destruction  
276 (Corenblit et al., 2014). During colonisation, *P. nigra* is known to enhance its local conditions  
277 (i.e., ecosystem engineer species) when growing at high seedling densities by trapping fine  
278 sediments and thus increasing nutrient availability and decreasing drought stress in its natural  
279 river reach habitat (Corenblit et al., 2014; Hortobágyi, Corenblit, Steiger & Peiry 2018). The  
280 modification of the environment by individuals can prevent, delay, or accelerate changes in the  
281 types of interactions between individuals (Corenblit et al., 2018). Based on the evolution of the  
282 SGS in a natural population, a shift in the type of interactions from positive to negative has  
283 been proposed in *P. nigra* stands along riparian corridors (Mazal et al., 2021; Mazal, Corenblit,  
284 Fumanal & Till-Bottraud 2022) (Fig.1). The case of *P. nigra* shows that, in long-lived species,  
285 shifts in the type of interaction are possible and could be monitored by studying SGS (Mazal,  
286 Corenblit, Fumanal & Till-Bottraud 2021). A cohort monitoring approach from establishment  
287 to maturity in long-lived species could be an efficient approach to follow the evolution of  
288 interactions between individuals and ultimately to estimate the survival component of fitness  
289 (not just reproduction). In addition, cohorts should also be followed in contrasted conditions, if  
290 possible along a gradient of environmental conditions, as the type of interaction may vary with  
291 life stages and environmental conditions. An increase in SGS over time could be an indication

292 of positive intraspecific interactions between relatives and thus would warrant further  
293 validation.

294         These examples open new research avenues to consider the impact of genetic variability,  
295 environmental conditions, life-span and SGS in kin selection studies. However, we believe that  
296 some semantic and methodological aspects need clarification to accurately address kin  
297 recognition, discrimination or selection in such studies.

### 299 **Kin recognition, kin discrimination and kin selection**

300 Kin recognition, kin discrimination and kin selection are often used alternatively in articles,  
301 however, they correspond to different concepts (see the review on kin recognition definitions  
302 by Penn & Frommen 2010). The confusion between kin recognition and kin discrimination  
303 probably comes from the fact that the only way to detect kin recognition in plants is through its  
304 expression (trait or behaviour variation between kin and non-kin neighbour treatments), i.e., kin  
305 discrimination. Indeed, many studies that showed the expression of a different phenotype  
306 depending on the genetic identity of the neighbours used the term kin recognition but *de facto*  
307 showed kin discrimination (Dudley & File 2007a, 2007b; Milla, Forero, Escudero & Iriondo  
308 2009; Murphy & Dudley 2009; Lepik, Abakumova, Zobel & Semchenko 2012; Semchenko,  
309 Saar & Lepik 2014; Yang, Li, Xu & Kong 2018; Li, Xu & Liu 2018; Goddard, Varga, John &  
310 Soulsbury 2020). Although kin discrimination can be captured by measuring any kind of trait  
311 that shows plasticity between kin and non-kin neighbours, its implication for kin selection can  
312 only be inferred if it translates into fitness differences between the two treatments (Ehlers &  
313 Bilde 2019). However, fitness is very rarely or only approximatively measured in “kin  
314 recognition” studies and some authors tend to extrapolate their results on kin recognition or  
315 discrimination and conclude without proof that there is evidence of kin selection in plants  
316 (Crepy & Casal 2015; Zhang et al., 2016; Pezzola, Pandolfi & Mancuso 2020).

317         Studies using biomass-related metrics (Fig. 2a) often suggest that reducing below-  
318 ground biomass is a cooperative behaviour indicative of kin selection. Increasing below-ground  
319 biomass when growing with neighbours (a competitive trait) diverts resources from producing  
320 more above-ground biomass. If the above-ground biomass is a good proxy for fitness  
321 (Younginger, Sirová, Cruzan & Ballhorn 2017), a cooperative decrease in below-ground  
322 biomass of all interacting individuals could thus increase their fitness (both direct and indirect  
323 if kin are involved). However, the relationship between biomass and fitness is not always

324 correct, especially in long-lived species such as trees, where biomass provides little information  
325 on how individuals will reproduce or may reflect many confounding factors, making their  
326 interpretation difficult (see the methodological issues discussed below and in Fig.2). In fact,  
327 results based on the observation of biomass differences can be interpreted in opposite ways  
328 (Subrahmaniam, Roby & Roux 2021). To our knowledge, the only article that came close to  
329 demonstrate a possible gain in fitness for closely related individuals (through the study of  
330 reproductive traits) is Torices et al. (2018). They showed that *Moricandia moricandioides*  
331 (Brassicaceae) individuals growing with kin produced larger floral displays than those growing  
332 with strangers. The production of a larger floral display is costly but attracts more pollinators  
333 that will also benefit their neighbours. The authors argue that individuals compensate for the  
334 decrease in direct fitness caused by the production of large flowers with a gain in indirect fitness  
335 by attracting more pollinators who will visit their related neighbours (Torices, Gómez & Pannell  
336 2018). In this case, related neighbours benefit from the attraction of pollinators that will  
337 pollinate their stigmas and export their pollen. These results were further supported by a second  
338 study which showed that plants growing with kin invest more in petal pigments, nectar volume  
339 and sugar content (Torices, DeSoto, Narbona, Gómez & Pannell, 2021).

340         Following the approach proposed by Ehlers & Bilde (2019), we recommend to move on  
341 to the next step and to study kin selection *per se* generalizing the use of Hamilton's rule ( $rB >$   
342  $C$ ), and the measurement of the cost ( $C$ ) and benefit ( $B$ ) components of fitness separately on  
343 each plant that take part in the interaction (note that, contrarily to what is stated in Ehlers &  
344 Bilde (2019), only *interacting* related individuals should be included for the estimation of  
345 indirect fitness), as well as their true (realized) relatedness ( $r$ ), which can now be reliably  
346 estimated using next-generation sequencing techniques (de Villemereuil, Gaggiotti, Mouterde  
347 & Till-Bottraud 2016). Two particular cases need to be detailed: if  $r = 0$ , no indirect fitness can  
348 be obtained. Conversely, if  $r = 1$ , Hamilton's rule is no longer needed to explain the outcome  
349 of the interactions because each interactant is actually a clone of the same individual and the  
350 indirect fitness is actually a direct fitness. This is the case in studies where clones are used as  
351 kin groups (Yamawo, Sato & Mukai 2017). The case of highly self-pollinating species (i.e.,  
352 same accession or variety) (Simonsen, Chow & Stinchcombe 2014; Yang, Li, Xu & Kong 2018;  
353 Pezzola, Pandolfi & Mancuso 2020) is slightly different because although very close to 1,  $r$  is  
354 actually slightly lower than 1. In a literature survey Subrahmaniam et al. (2021) showed that  
355 44% and 10% of the studies that investigated interactions between related and unrelated  
356 individuals were conducted in self-pollinating or clonal species, respectively. Can we extend

357 the results of these studies to other species that are not self-pollinated, and how general is a  
358 mechanism if it is only shown to work for clonal or self-pollinating species? Although the cases  
359 where  $r$  is very close to 1 are convenient to study, we think that they are rather extreme cases  
360 and that they are therefore difficult to generalize to other species. In natural populations without  
361 inbreeding, the expected relatedness full-sibs is 0.25, and values measured for ants and bees  
362 where kin selection has been demonstrated range from 0.5 to 0.7 (Gadagkar et al. 2019; Kay,  
363 Lehmann & Keller 2019). We argue that results obtained from highly-selfing or clonal species  
364 should be interpreted in the same way as studies on self-recognition (i.e. recognition of  
365 individual genotype between two ramets, be they connected or disconnected) (see also Bilas,  
366 Bretman & Bennett 2021).

367         As underlined by Hamilton's rule, the level of relatedness between individuals is critical  
368 to predict the outcome of the interactions. In a local population, varying levels of genetic  
369 relatedness can co-exist and thus patterns of interactions between individuals can vary based on  
370 their relatedness (Subrahmaniam, Roby & Roux 2021). Following Hamilton's rule, the potential  
371 benefits of interaction should decrease linearly or display a threshold (Till-Bottraud & de  
372 Villemereuil 2016) beyond which it is no longer advantageous to cooperate ( $C > rB$ ). However,  
373 the gradient of relatedness between individuals is rarely addressed in studies and this raises the  
374 question of the generalized use of studies that tested interactions between individuals  
375 originating from a single population (Subrahmaniam, Roby & Roux 2021). Few studies thus  
376 far have used different levels of relatedness between individuals. Karban et al. (2014), for  
377 example, used a gradient of relatedness and found that *Artemisia tridentata* individuals  
378 responded more effectively to volatile cues from close relatives than from distant relatives when  
379 subjected to herbivory. Another study using rice cultivars with different levels of relatedness  
380 showed that plants altered their root behaviour and biomass allocation in response to relatedness  
381 level (Yang, Li, Xu & Kong 2018). Moreover, using a relatedness gradient, Xu et al., (2021)  
382 showed that plants discriminate their neighbours (related or unrelated) and adjust their growth,  
383 competitiveness and chemical defence accordingly to improved weed suppression in rice  
384 (through allelopathic processes) (see also commentary from Anten & Chen 2021b). Following  
385 on the authors' discussion, we also believe that a relatedness gradient is an important factor that  
386 can potentially trigger responses when plants interact with neighbouring individuals and thus  
387 must be considered in future studies.

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### 392 **Methodological challenges in studying kin recognition and selection**

393 Whether studies did (see the review by Anten & Chen 2021a) or did not find evidence of kin  
394 recognition (e.g., Milla, Forero, Escudero & Iriondo 2009; Lepik, Abakumova, Zobel &  
395 Semchenko 2012), much caution in their interpretations must be taken. When comparing  
396 biomass of plants growing with kin or strangers, alternative hypotheses and several  
397 confounding factors could explain the results and should be tested. A common difficulty to find  
398 evidence of kin recognition in experiments is the lack of appropriate control treatments (Fig.2b).  
399 In pot experiments, the use of a control treatment where plants are grown alone is mandatory  
400 to determine if plants do compete or not (compared to plants grown alone). This control  
401 treatment is also necessary to detect a potential genotype that has a better intrinsic growth.  
402 Genotypes with better intrinsic growth capacities can lead to size inequality in groups of plants  
403 (Simonsen, Chow & Stinchcombe 2014; Li, Xu & Liu 2018). Another confounding factor is  
404 competitive ability, i.e. the relative growth of individuals grown in competition compared to  
405 when grown alone (Masclaux et al., 2010; Zhang et al., 2019). Although it is usually argued  
406 that a species can win either through being better at suppressing the performance of others  
407 (effect) or being better at tolerating competition (response), Hart et al. (2018) showed that only  
408 the competitive response is relevant to a species' competitive ability. Following early works on  
409 kin recognition and discrimination in plants, several papers encouraged testing for competitive  
410 ability (Masclaux et al., 2010; Chen, Doring & Anten 2012). However, this is still not a standard  
411 procedure across studies and the lack of appropriate control treatments remains common in the  
412 literature (Semchenko Saar & Lepik 2014; Crepy&Casal 2015; Murphy et al., 2017; Murphy,  
413 Swanton, Van Acker & Dudley 2017; Li, Xu & Feng 2018; Pezzola, Pandolfi & Mancuso  
414 2020). Here, we would like to emphasise that this lack of control treatment may result not only  
415 in misinterpretation but also in the failure to reveal kin discrimination in studies (that have  
416 therefore not been published). To test for competitive ability, it is necessary to set all the kin  
417 groups in competition with each other and test whether a kin group always performs better than  
418 the others prior to wrongly conclude to kin discrimination.

419 When plants grown with relatives were found to exhibit greater belowground biomass  
420 than plants grown with strangers, the results were mostly interpreted using the niche partitioning  
421 framework (File, Murphy & Dudley 2012; Subrahmaniam, Roby & Roux 2021). Niche

422 partitioning predicts that relatives overlap more in their niche use and compete more with each  
423 other compared with unrelated conspecifics (Young 1981; Milla, Forero, Escudero & Iriondo  
424 2009). However, stronger competition between relatives can be a by-product of forced  
425 competition for a few dimensions of the niche that occurs when plants are grown at high density  
426 in pots. The niche is a multidimensional concept (Hutchinson 1957; González, Dézerald,  
427 Marquet, Romero & Srivastava 2017) with each component (e.g., light, nutrient, water, wind,  
428 space, microbiota) occupying one dimension. Pots strongly constrain the growth of roots for  
429 example in space or for the types of nutrients available. In pots, individuals share and compete  
430 in a restricted number of dimensions and there may be no possible compensation in other  
431 dimensions whereas in nature, individuals can compete in some dimensions of their niche but  
432 not in others (Barker et al., 2017). Pot size can thus obscure the effects of positive interactions  
433 between individuals (Hess & de Kroon 2007; Chen, Daring & Anten 2012) because root growth  
434 responds to the available volume (McConnaughay & Bazzaz 1991), but also to nutrient amounts  
435 (O'Brien, Gersani & Brown 2005) and concentrations (Maina, Brown & Gersani 2002).  
436 Therefore, experiments conducted in restricted conditions (i.e., few niche dimensions) will bias  
437 the conclusions towards niche partitioning versus kin discrimination and would not be relevant  
438 in natural conditions where individuals growing with relatives can exploit all the dimensions of  
439 their niche. On the other hand, when plants are supplemented with nutrients and resources are  
440 not limiting, one can expect to find little or no difference between the stranger and kin  
441 treatments because every individual has access to sufficient resources for its growth. Finally,  
442 kin cooperation (kin selection) and competition (niche partitioning) can occur simultaneously,  
443 possibly acting on different traits and thus different niche components (File, Murphy & Dudley  
444 2012). Thus, depending on the observed traits and growth conditions it is possible to observe  
445 one process but to miss the second. Therefore, although *in situ* experiments are challenging, we  
446 share Anten & Chen's (2021a) appreciation that *in situ* experiments could solve some of the  
447 problems associated with experiments under controlled conditions, in particular the constrained  
448 niche aspects.

449

## 450 **Conclusion and final notes**

451 Over the last decade the scientific literature has continuously explored the role of relatedness  
452 in the interactions between plants. However, we assert that some points have been generally  
453 neglected and are worth considering in kin recognition/selection studies. The major aspects are  
454 the underestimated role of intraspecific diversity for kin recognition and kin selection traits and



455 the fact that local conditions can influence the outcome of interactions. The concept of  
456 "conditionality" and the "stress gradient hypothesis" suggest that intra-specific interactions, and  
457 in particular interactions between relatives, are dependent on environmental conditions,  
458 especially under various stress types. The case of long-lived species, where individuals face  
459 different environmental conditions and stresses throughout their life, is especially promising.  
460 In addition, studying the spatial genetic structure of populations for *in situ* studies would be of  
461 great help in pointing out settings in which different types of interactions might take place. We  
462 also discuss the challenges of distinguishing kin discrimination from kin selection, which are  
463 to accurately measure the costs and benefits of the interaction as well as the relatedness of the  
464 interacting individuals, and the importance of appropriate control treatments to test for all  
465 alternative hypotheses (Fig. 2). We also strongly recommend several key-points to be  
466 considered in future studies (Fig. 2b): to capture a large intraspecific diversity of the studied  
467 species, to include different environmental conditions and to include a relatedness gradient  
468 between individuals. This would help to take care of various confounding effects that could  
469 interfere with the interpretation of the results.

470

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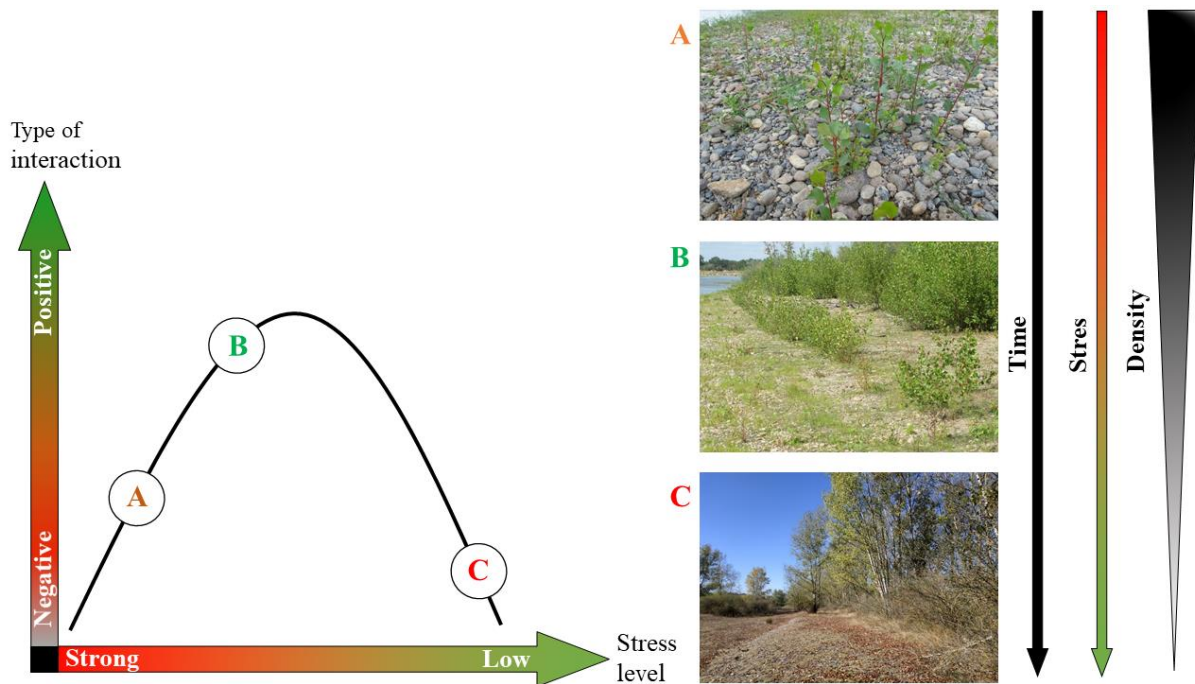
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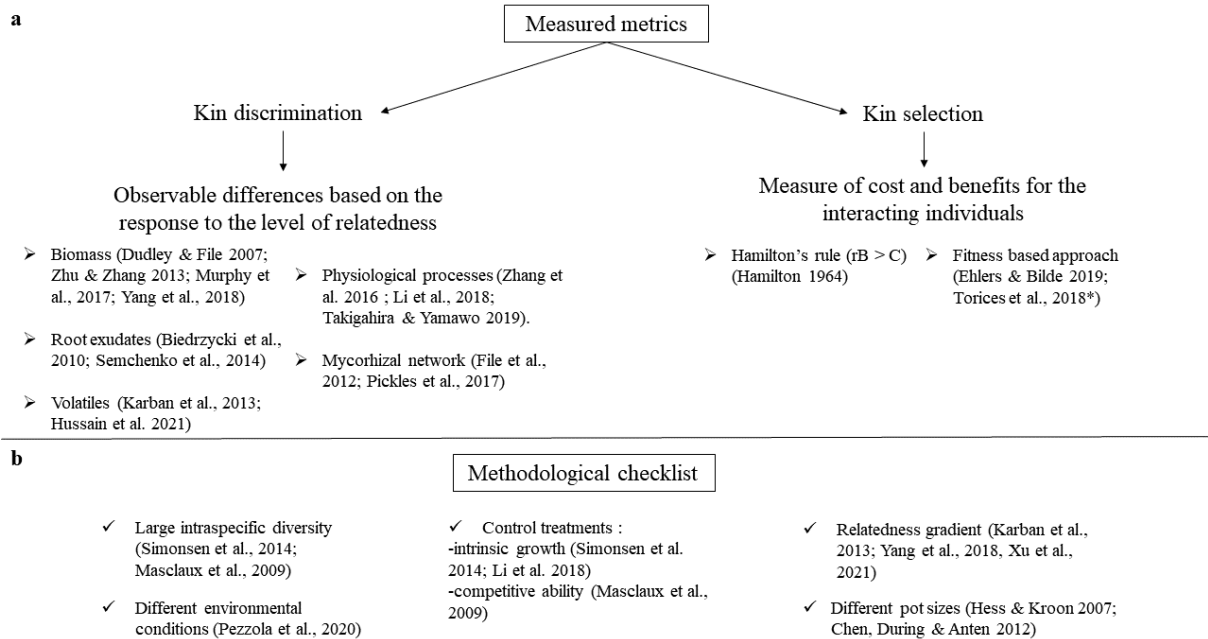
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## 710 Figures



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712 **Figure 1.** The stress gradient hypothesis (SGH, Bertness & Callaway, 1994) applied to the case  
713 of *Populus nigra*. A: Seedlings establish in densities on bare surfaces along rivers and  
714 experience harsh conditions (mechanical and hydric stresses). Facilitation occurs due to the  
715 high densities of individuals and reduces the stresses for each individual. At this stage no spatial  
716 genetic structure (SGS) is found and the main direct interactions between individuals are neutral  
717 (commensalism). B: Patches of young individuals that survived now exhibit SGS. The effect of  
718 *P. nigra* on the environment enhance the local conditions (soil moisture and nutrients).  
719 Facilitation still occurs because of the protective effect of individuals located at the upstream  
720 part of the stand (Mazal et al., 2021). Main direct interactions shift to positive (potentially  
721 cooperation) and could be directed towards related individuals (as SGS is significant at this  
722 stage). C: At older stages, individuals no longer benefit from the presence of their neighbours  
723 and the local conditions are no longer stressful. Stem density is highly reduced through self -  
724 thinning processes (i.e., competition) (Yoda et al., 1963). The main interactions between  
725 individual are either neutral (commensalism) or negative (competition).



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727

728 Figure 2. Suggested guidelines for the design of experiments on kinship studies in plants. (a)  
729 Measured metrics commonly used in the conceptual frameworks studied: kin recognition  
730 discrimination or kin selection. (b) Checklist for the design of future studies outlining key  
731 points to be controlled. For both (a) and (b), we have mentioned the studies that in our opinion  
732 have taken some of the points above into account and that could be used as a reference. \*As  
733 discussed in the text, although this study did not measure the fitness of individuals, it is the  
734 closest evidence to date for kin selection in plants.  
735