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1 Original article

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4 Phenotypic diversity assessment within a major ex situ collection of coffees in Madagascar

5

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23

1 ABSTRACT

2

3 *Background and aims.*

4 Like other clades, the *Coffea* genus is highly diversified on the island of Madagascar. The 66
5 endemic species have colonized various environments and consequently exhibit a wide
6 diversity of morphological, functional, phenological features and reproductive strategies. The
7 trends of interspecific trait variation, which stems from interactions between genetically
8 defined species and their environment, still needed to be addressed for Malagasy coffee trees.

9 *Methods.*

10 The structure of endemic wild coffees was explored in terms of morphological, phenological,
11 and functional traits. The environmental (natural habitat) effect was assessed on traits in
12 species in distinct natural habitats. Phylogenetic signal (Pagel's λ , Blomberg's K) was used to
13 quantify trait proximities among species according to their phylogenetic relatedness.

14 *Key result.*

15 Phylogenetic signal was found to vary greatly across and even within trait categories. The
16 highest values being exhibited by the ratio of internode mass to leaf mass, the length of the
17 maturation phase and the LDMC. By contrast, traits weakly linked to phylogeny were either
18 constrained by environment (leaf size) or under selective pressures.

19 *Conclusions*

20 This study brings insight into complex patterns of trait variability. The length of the
21 maturation phase, an adaptive trait, shows a strong phylogenetic signal as does the ratio
22 between the stem dry mass and leaf dry mass.

23

24 Keywords: phenology, LMA, LDMC, functional traits, *Coffea*, Rubiaceae

25

1 INTRODUCTION

2

3 Tropical rainforests of Africa and Madagascar harbor huge biodiversity characterized by great
4 species richness and endemism (Myers, 2000; Yoder and Novak 2006, Vences et al. 2009).

5 In such context, for the genera that experienced radiative speciation, phenotypic divergence
6 can be important while the associated genetic differentiation could remain low resulting in

7 low congruency between genetic and phenotypic diversity, and in the recognition of
8 morphological units as species (Shaffer and Thomson, 2007). For instance, 58 and 47 *Coffea*

9 species (excluding *ex-Psilanthus* genus and *Baracoffea* grouping) respectively from
10 Madagascar and Africa were described (Davis et al. 2006, Couturon et al. 2016), although

11 three and seven species from Madagascar and Africa respectively are not yet formally
12 recognized.

13 For Coffee, clear relationships between species was not permitted by analysis conducted with
14 two ITS plus four plastid sequences (Maurin et al. 2007), nor with a limited number of

15 nuclear microsatellite markers (Razafinarivo et al. 2013). A completely resolved molecular
16 phylogenetic tree was obtained using 28,800 concatenated SNPs obtained from Genotyping-

17 By-Sequencing (GBS) methodology (Hamon et al. 2017). The necessity to use GBS for
18 assessment of between-species genetic divergence could be explained by two non-exclusive

19 mechanisms: 1) the absence of strong genetic barrier and therefore the maintenance of gene
20 flow between species even at a low rate (Hey 2006, Nosil 2008, Niemiller et al 2008) and 2)

21 recent speciation coupled with rapid morphological divergence (Janssen et al. 2008).
22 Regarding Madagascar speciation, the general agreement states that “the importance of

23 dispersal to the assembly of the Malagasy flora cannot be denied”(Yoder and Nowak, 2006).
24 The high level of speciation seems to be associated with the existence of different small

1 niches side-by-side. Therefore, the resulting species should be highly differentiated at the
2 phenotypic level but not, or less, at the genetic one (Yoder and Nowak, 2006).

3

4 In addition to environmental selective pressures, traits (i.e. individual heritable features, as
5 defined by Garnier and Navas 2012) are also shaped by developmental (i.e. structural
6 limitation on phenotypic variability, as defined by Smith *et al.* 1985), functional and genetic
7 constraints.

8 Common evolutionary origin or, at the opposite, evolutionary convergence could be the result
9 of similar trait values for two species, or of correlations between traits. Hence, functionally
10 similar species may belong to the same clade or may be phylogenetically distant. Regarding
11 leaf functional traits such LMA, leaf mass per area, and LDMC (ratio dry leaf mass/fresh leaf
12 mass); high LMA values characterize plants with low growth producing large leaves but
13 whose longevity is low (Westoby et al 2002); high LDMC values characterize plants whose
14 foliar organs accumulate nutrients and constitute important storage pools with low tissue
15 turnover. Therefore, the study of their relationship permits to test whether there is
16 compensatory effect between these two traits and whether this correlation still exists in case
17 of evolutionary trade off.

18 The most recent *Coffea* molecular phylogeny highlighted independent and parallel
19 diversification in the two landmasses Africa and Madagascar (Hamon et al. 2017) resulting in
20 total endemism in these main regions. More widely, none of the species occurring in West
21 and Central Africa is found in East Africa, in the Western Indian Ocean Islands, nor in Asia
22 (broad sense) and conversely. Another important characteristic concerns the great
23 vulnerability of *Coffea* species, as their distribution is generally restricted to one or a few
24 forests (Davis et al., 2006; <http://publish.plantnet-project.org/project/wildcofdb>). In such
25 context, forest cutovers and burns, human activities, and natural hazard (cyclones, fires) could

1 dramatically decrease *Coffea* richness both at the population and species levels. As a
2 consequence, more than 80% of *Coffea* species are present on the red list of endangered
3 species of the IUCN (from nearly threatened to critically endangered, Davis et al. 2006). Yet,
4 wild Madagascan species constitute highly valuable genetic resources (Andrianasolo et al.
5 2013). A large part of the Malagasy species are grouped together in an ex situ collection. Such
6 collection is especially important for coffees since some of them have already disappeared
7 from their natural habitat (Krishnan et al. 2013). As part of the study, we were able to carry
8 out *in situ* and *ex situ* comparisons of genetic diversity for four species and two populations
9 per species. This study showed a strong genetic diversity in the *ex situ* collection
10 (Andrianasolo et al., 2013). The plants in collection are very valuable for studies on
11 interspecific variability.

12

13 Madagascan *Coffea* show different growth habits, growing as ligneous shrubs, treelets or
14 trees. They are mainly plants, with only monopodial growth, axillary inflorescences and
15 evergreen leaves (66 species at all; Davis *et al.*, 2006, Couturon *et al.*, 2016,
16 <http://publish.plantnet-project.org/project/wildcofdb>). But some species have a sympodial
17 growth (Davis and Rakotonasolo, 2008). Most *Coffea* species conform to the Roux's
18 architectural model with rhythmic growth and continuous branching, and lateral flowering on
19 the plagiotropic branches (Hallé et al., 1978). In *Coffea* species, stem growth occurs phytomer
20 by phytomer, with a phyllochron that varies depending on seasonality (Varossieau, 1940).
21 Another striking example of adaptation lies in their phenological traits. For all species,
22 inflorescence and flower development require the dry season to be followed by a an adequate
23 rainfall (Portères, 1946). The number of days from this triggering rain to the opening of the
24 flower bud (anthesis) is genetically determined and depends on the species considered (Le
25 Pierrès, 1995; Noiroto et al, 2016). A better knowledge of the reproductive strategies is an

1 important first step for the development of appropriate conservation strategies. The length of
2 the maturation phase which correspond to the time between the seed emission and the seed
3 germination is very variable between species according to the plant habitat.

4

5 Within the past few years, as more phylogenetic data got available, a number of studies have
6 connected phylogenetic analyses with phenotypic, physiological, or phenological traits
7 (Reginato and Michelangi, 2016; Bacon *et al.*, 2016). Several descriptive statistics have been
8 introduced to gauge phylogenetic signal as a quantity that could thus help compare different
9 traits across phylogenies (Pagel 1999; Blomberg *et al.* 2003). Not all traits behave similarly
10 for closely-related species: while some traits stay stable throughout the phylogeny (Davies *et*
11 *al.*, 2013), others are highly labile (Cavender-Bares *et al.*, 2009). Owing to shared ancestry,
12 non-independence caused by evolutionary history is expected from species traits.
13 Phylogenetic signal measures this tendency for related species (Blomberg and Garland, 2002):
14 the stronger the signal, the larger the influence of the phylogenetic structure on the species
15 traits.

16

17 In this study, the availability of *Coffea* molecular phylogeny and phenotypic data for a large
18 set of Madagascan wild coffees allows us to address the following questions: How is the
19 phenotypic variation distributed among Madagascan wild coffee species and which are their
20 phenotypic relationships? Are there different reproductive strategies? Are there phenotypic
21 traits associated with species evolution, and thus expressing phylogenetic signal?

22

23

24 MATERIALS AND METHODS

25

1 *Plant Material*

2 Plant material comes from the *ex situ Coffea* collection at the Kianjavato Coffee Research
3 Station (KCRS) in Madagascar, (coordinates S 21°22'28", E 47°52'02"). The collection is
4 situated 550 km away from Antananarivo, at altitudes ranging from 50 to 400 m above sea
5 level. The collection is mainly constituted by Madagascan wild *Coffea* species (Chevalier,
6 1942) excluding the *Baracoffea* group from the Western coast described by Davis and
7 Rakotonasolo (2008). They are representatives of the five botanical series from the Great
8 Island, which were classified by Charrier (1978) according to floral and leaf traits. These
9 series are named Garcinioides, Millotii complex, Multiflorae, Subterminales and Verae.
10 The coffee trees sampled are adult trees mainly collected between 1960s and 1980s, growing
11 under natural forest cover. Details on plant material (such as species name, botanical series,
12 geographic origin, and coordinates) are provided in Table 1.

13

14 *Phenotypic records*

15 Records were done during years 2010-2011, 2013-2014 on a total of 364 plants from 63
16 populations corresponding to 36 species. Descriptive (such as, leaf shape and color, domatia
17 position, aperture shape and pubescence) and quantitative traits on the numbers, leaves, fruits,
18 seeds size and phenology (number of days between the triggering rain and blossoming,
19 between flowering and ripening, number of flowers per inflorescence) were recorded.
20 Excepting for leaves, fruits and seeds measurements, the trait records were done on a
21 maximum of ten trees (depending on the availability) per population.

22 Mature leaves measurements were performed on five leaves (picked on the fifth branching
23 from the top) and five trees per population.

24 Measurements for fruits (including 100-seed weight and fruit disc diameter) were done on 20
25 and 40 seeds and fruits respectively per population. Four additional variables were calculated:

1 i) the shape index of leaves as the ratio of length to width; ii) the surface index as length x
2 width; volume indices of iii) fruits and iv) seeds as length x width x thickness. The 28
3 variables and range of variation are listed in Table S1.

4 The number of days for maximum seed germination was obtained after sowing between 68
5 and 120 seeds per harvest in nurseries at Kianjavato. This trait was recorded for 47
6 populations corresponding to 28 species.

7 Two functional attributes, leaf mass per area (LMA, recorded for 39 populations either 33
8 species) and leaf dry matter content (LDMC, i.e. the ratio of leaf dry mass to leaf fresh mass)
9 which was recorded for 30 populations either 29 species, were added, as well as two
10 physiological attributes: stomata density and stomata length, as measured in Razafinarivo et
11 al. (2012) were recorded for 56 populations either 35 species. The Leaver module was used to
12 extract leaf area with the plugin Toaster (Tree and plant organs and structures analyzer,
13 Borianne, 2012) run in the ImageJ software. The leaf dry mass was obtained after being dried
14 four days in the oven (60°C). For all species, in order to compare even-aged organs, the
15 measurements of size leaf and internode have been made on the more recent metamer at the
16 branch extremity and in full light. These metamer were green and without secondary growth.

17

18 *Environmental parameters*

19 Climatic data were extracted from GPS coordinates of each species (Table 1) and from
20 worldclim information (<http://www.worldclim.org>). The “habitat class”, (Table 1) is obtained
21 from a distribution of the number of dry months of each species original habitat.

22 Among the 19 available bioclimatic variables, five (Annual Mean Temperature, Max
23 Temperature of Warmest Month, Min Temperature of Coldest Month and Annual
24 Precipitation) were retained for this study. Additional climate data such as Annual Potential
25 evapotranspiration, Climatic Water Deficit and, Number of Dry Months were extracted from

1 MadaClim (<https://madaclim.cirad.fr/>). An Index of Aridity was also calculated as annual
2 precipitation minus annual potential evapotranspiration (Table 2).

3

4 **Statistical analyses**

5 *Relationships among Madagascan wild coffees*

6 Given that the recorded traits are quantitative and qualitative, quantitative traits (at all 14)
7 were transformed into either similar size classes or well distinct range classes depending on
8 the distribution of the trait. For the qualitative trait, each modality became a new trait.
9 Therefore, from the 28 initial variables, a total of 107 new variables were obtained and
10 encoded 1-0 for presence-absence. With the aim to assess the relationships between
11 individuals within populations, populations within species and between species, a Neighbour
12 Joining (NJ) tree based on matrix of Dice's dissimilarities indices and 200 bootstraps was
13 constructed using DARwin v.5 (Perrier and Jacquemoud-Collet, 2006).

14 For quantitative traits (reproductive, vegetative, phenological, physiological and functional
15 leaf traits), we used standardized data to visualize for each trait and each species the deviation
16 to the mean of the values obtained for all the species. The results for each species are ordered
17 following the rooted molecular phylogenetic tree extracted from Hamon et al. (2017).

18

19 *Relationships between phenotypic and bioclimatic traits*

20 **Three habitat classes of species** were defined according to the number of dry months from the
21 original population location: 0-1 dry month for S1, 2-6 dry months for S2 and >6 months for
22 S3. Comparisons were performed on seven traits recorded for all populations investigated **(63**
23 **populations corresponding to 36 species)**. For 15 traits, data were obtained for 54 to 61
24 populations. Six traits were recorded for 30 to 39 populations corresponding to at least 29

1 species. Mean comparisons between subsets were performed with ANOVA using R (R Core
2 Team, 2015).

3

4 *Analysis of interspecific and intra specific (population) diversity*

5 We summarized the interspecific and intraspecific (population) trait variation in the table S2
6 (Supplementary material), showing the mean trait values for a set of species. Differences
7 between populations were analyzed by one-way ANOVA and the Tukey test. We also verified
8 the contribution of the population level to the total trait variation by decomposing the variance
9 of each trait using a linear mixed model that took into account the nested structure of the
10 sampling design (package lme4, Bates *et al.*, 2015)..

11

12 *The phylogenetic signal*

13 The phylogenetic signal allows a better understanding of the evolutionary traits within a
14 group. The phylogenetic Pagel's λ (Pagel, 1999) and Blomberg's K indices (Blomberg *et al.*,
15 2003) were selected after assessment of their respective performance and shortcomings.
16 Pagel's λ estimated by a maximum-likelihood approach provides the most probable
17 description of the observed trait distribution given Brownian model for trait evolution (BM).
18 A value of λ equal to 1 indicates that the trait evolves exactly as suggested from the original
19 topology of phylogeny while a value of λ equal to 0 means an absence of phylogenetic signal.
20 Blomberg's statistic (K) quantifies phylogenetic signal. It is a descriptive statistical parameter
21 to describe the degree of the difference between the *F*-statistic of simulated data and observed
22 *F*-statistic distribution by the ratio of the observed mean squared error derived from a
23 phylogenetically corrected mean and the expected mean square error obtained from the
24 analysis by considering tree topology and branch length information. A K value inferior to
25 one suggests that the trait evolution was independent of phylogeny (*i.e.* the phylogenetic

1 signal is low). A K value equal to 1 suggests that the trait has evolved at a constant rate during
2 species evolution, while $K > 1$ indicates a higher phylogenetic signal for the considered trait
3 (*i.e.* the trait is more similar for related species than expected by the evolutionary model). The
4 significance of K is obtained from 999 permutations. Phylogenetic signal was only considered
5 significant for a trait when both Pagel's λ and Blomberg's K gave consistent results with the
6 following thresholds for the p-values: $p_\lambda < 0.05$ and $p_K < 0.1$. Phylogenetic signals were
7 estimated with the function `phylosig` in package 'phytools' (Revell, 2012) from mean values
8 data per species for 29 to 36 species depending on the trait.

9 Statistical tests (normality, independence and homoscedasticity of residuals) for validation of
10 the linear model were performed using package `lmtest` (Zeileis and Hothorn, 2002).
11 Calculations were performed with the function `pgls` of the R package 'caper' (Orme *et al.*,
12 2012). Figures drawings are performed within R 3.2.5.

13

14 RESULTS

15 *Structuring of wild coffees based on traits*

16 Individual phenotypic relationships showed that globally, intraspecific variation was lower
17 than interspecific variation, which can be seen in the table S2 (Supplementary material) with
18 ANOVA results on interspecific variation compared to intraspecific (population) variation.
19 The deviation to the mean was lower for intraspecific variation, and populations were
20 generally pooled into fewer groups than did species. The contribution of intraspecific
21 diversity to the variance of species traits (table S3, Supplementary material) varied between
22 traits, being fairly high (leaf length , number of flowers per inflorescence), moderate (shape
23 index, petiole length) or low (length of the maturation phase).

24

1 The estimation of dissimilarity indices within populations lower than between species (Figure
2 1). As an example, *C. resinosa* individuals were grouped close to each another, even if they
3 are exhibiting an important phenotypic variability.

4 Only two Charrier's series (the Millotii complex and the Garcinioides) appeared very
5 homogenous but no overall structuring according to the botanical series classification is
6 obvious. Using standardized data for each trait permitted to discern some trends following
7 phylogenetic species relationships (Figure 2). The ratio between internode mass and leaf mass
8 was higher for three species: *C. vohemarensis* and *C. vatovavyensis* (Subterminale serie) and
9 *C. bertrandii* (Multiflorae serie) that are highly branched and bear small leaves.

10 Another grouping of sister species (Figure 2, in red) was characterized by long petiole and
11 leaves, thick and long internodes, large leaf area, large fruits and seeds (Figure 3A). Similarly,
12 a grouping of northern species (Figure 2, in yellow) was characterized by a long maturation
13 phase and included two species easy to identify from their fruit shape (*C. tsirananae*, Figure
14 3B) or their leaf shape (*C. heimii*, Figure 3C). The latter species also exhibited the highest
15 value for LMA.

16 *Reproductive traits and strategies*

17 Madagascan wild coffee trees exhibited a wide range of number of days between the
18 triggering rain and the flowering (from 4 to 13 days). Two series, Garcinioides and Millotii
19 complex, were characterized by a long duration from rainfall to flowering (10-13 days) while
20 the Verae exhibited a short to medium duration (4-7 days). Regarding the three bioclimatic
21 subsets of species S1, S2 and S3, the range of the number of days between the trigger rain and
22 blossoming was large for the three subsets going from four to 10 (S2) or 13 (S1) days and,
23 from seven to 12 for S3 (Figure 4A). The Multiflorae appeared as the most diverse series with
24 from short (4 days) to long (10 days) durations (Figure 4B).

1 Maturation phase lengths were also diverse and ranged from 59 to more than 350 days. In
2 very humid habitats (0 to 1 dry month) as well as in intermediate habitats (2 to 6 dry months),
3 species ripen in 2 to 6 months. In very dry habitats (>6 dry months), species ripened in
4 contrasted time, either 2-3 months (for the majority), either around one year (Figure 5A).
5 Dates for harvest went around the year from January to December (Figure 5B). Early species
6 (all Verae, and majority of Subterminales and Multiflorae) were harvested from January to
7 February. Medium species (mainly Millotii) were harvested from March to May and very late
8 species (all Garcinioides) from mid-November to December. The sequence of harvest was
9 nearly invariable whatever the year. Considering seed germination; generally there was either
10 no dormancy or germination after a couple of months.

11 In humid (S1) and intermediate (S2) habitats, maturation phase and seed germination took
12 place during the hot rainy season with short time between the two physiological steps.
13 However, in dry habitats (S3), two behaviors were found. Either species (such as *C.*
14 *boiviniana*) had a very short maturation phase followed by seed germination few weeks after,
15 either species such as *C. tsirananae* had a long maturation phase (around one year) followed
16 by immediate seed germination (occurring during the rainy season at year n+1 after the
17 flowering). An overview of the timing of the different reproductive phases considered as
18 reproductive strategies is given in Figure 6.

19

20 *Environmental effect*

21 **Given the distribution of the number of dry months in their natural habitats, three habitat class**
22 **of species were defined from the bioclimatic parameters of their natural habitat.** The main
23 features of these habitat class of species are given in Table 2.
24 Higher the number of dry months, higher the range of the average temperature of the coldest
25 month and higher the annual potential evapotranspiration as a result of warm temperature.

1 Regarding biological trait, mean comparisons between habitat class for each trait showed
2 significant differences between the three habitat class as described in Table 3 for vegetative
3 traits (leaf length, leaf width and leaf area, two leaves mass and internode diameter
4 perpendicular to leaves, ratio of internode mass/two leaves mass, ratio of internode
5 length/diameter, Ratio Length of internode/internode diameter parallel to leaves), and
6 reproductive traits (number of flowers per inflorescence, maturation phase length, and seed
7 thickness).

8

9 *Phylogenetic signal*

10 The phylogenetic signal allows a better understanding of the evolutionary traits within a
11 group. Phylogenetical signal was assessed using Pagels' λ and Blomberg's K parameters to
12 describe the degree of the difference between the F -statistic of simulated data obtained from
13 999 permutations and observed F -statistic distributions.

14 We found that 9 traits (ratio of internode mass/leaf mass, petiole length, parallel internode
15 diameter, ratio of internode length/diameter, leaf length, the length of the maturation phase,
16 seed shape index, LDMC, stomata length and stomata density) evolved in relation with
17 species evolution (Figure 7).

18

19 DISCUSSION

20 *Reproductive strategies and limitation of gene flow*

21 Our study showed that phenology parameters (the number of days for blossoming after the
22 trigger rain and the maturation phase length, see Fig 4 and 5) did not correlate with natural
23 habitat type; similar range of variation was observed in the three types. For coffees, flowering
24 requires two important steps. The first one is the buds inducing that is set up after a dry
25 period. It is climate dependent as was reported in New-Caledonia by Gomez et al. (2016).

1 Indeed, flowering patterns for the three cultivated *Coffea* species (*C. canephora*, *C. liberica*
2 and *C. arabica*) introduced during the beginning of 19 century followed the timing and
3 sequence of precipitation in the study area. The second step corresponds to the development
4 of the flower buds following a trigger rain (of at least 10 mm, Portères, 1946). It is genetically
5 determined since the average number of days between the trigger rain and the blossoming is
6 fixed for each species and ranged here between 5 and 13 days (Noirot et al., 2016). These
7 values remained invariable whatever the growing place as was observed in New-Caledonia
8 for the three introduced species (Gomez et al. 2016). In such conditions, climate changes or
9 new climate growing environment should affect only buds inducing and then, as a
10 consequence, the quality of blossoming that depends on the intensity of the trigger rain. Under
11 climatic changes, flowering could be disturbed. Erratic flowerings might open the way for
12 flowering synchronization of usually non-synchronous flowering species and thus favoring
13 unusual gene flow. However, the loss of species integrity involve also cross-species
14 hybridization success and capability to produce fertile progenies. This phenomenon is
15 probably at the origin of the natural cross between two wild species, producing the famous
16 Arabica coffee (Carvalho, 1952; Lashermes et al, 1999).

17

18 Within tropical forests, a broad spectrum of flowering patterns has been found (Sakai 2001),
19 and observed variations in phenology may arise from phenotypic plasticity or from
20 genetically based local adaptations or a combination thereof (e.g., Zalamea et al. 2011;
21 Anderson et al. 2012). Coffee flowering phenology has been characterized as highly
22 dependent on climatic factors such as precipitation (Crisosto et al. 1992), and temperature
23 (Lin 2008), and it is genetically controlled (Le Pierrès 1995). The time between this rain and
24 the flowering date was well conserved, strictly species-dependent and followed a clear

1 sequence in which *C. liberica* flowered first, followed by *C. canephora* and then *C. arabica*
2 both in Africa and New-Caledonia (Gomez et al. 2016).

3 For Madagascan wild coffees, the sequence of flowering is also well-conserved starting in
4 September by *C. sp.* ‘Sambava-Diego’ (described in Couturon et al. 2016 and in
5 <http://publish.plantnet-project.org/project/wildcofdb>) and *C. vatovavyensis*. This is followed
6 in October by a part of Subterminales, Millotii complex and Multiflorae species, followed in
7 November by the late-flowering species belonging to Millotii complex and Multiflorae, all the
8 Garcinioides and *C. boiviniana*, *C. alleizettii*, *C. augagneuri*, *C. ratsimamangae* and *C.*
9 *sakarahaie* (Subterminales) and finishing in December with the very late-flowering *C.*
10 *tsirananae* (Subterminales).

11 Flowering patterns in *Coffea* thus appear to have a strong genetic component, causing the
12 flowering times of each species to remain distinct (Akaffou et al. 2014). Partly asynchronous
13 responses among species to key environmental factors would thus be expected to act as
14 prezygotic barriers. The lack of a well-marked dry season is considered to influence flower
15 bud formation by creating different levels of induction (Crisosto et al. 1992) and favoring
16 multiple flowering events during a season (Allan, E., and J. R. Pannell. 2009). However, we
17 do not know whether adaptive evolution will allow populations to reach new phenotypic
18 optima rapidly enough to keep pace with climate change (Anderson et al., 2012).

19

20 Reproductive barriers due to no overlap of flowering is very important for sympatric species.

21 This is all the more important given that hand-cross fertilizations in coffees were done in
22 Ivory Coast and in Madagascar (Louarn 1992, Charrier 1978). Despite differences in F1
23 hybrids and F1 fertility rates, the authors concluded that coffees share the same nuclear
24 genome. Therefore, in natural conditions, species integrity in sympatry conditions should be
25 mainly maintained by no flowering overlapping. So, the differences in days between the

1 trigger rain and blossoming are an essential component. However, this physiologic barrier is
2 not absolute. Buds inducing is highly dependent on climate parameters (alternating dry and
3 rainy periods). Reproductive strategies involve mechanisms that favor species integrity
4 (physiologic flow gene barrier), and species survival in climate changes and during new niche
5 colonization.

6 For species survival, legitimate seed germination must benefit from rains to produce plantlets
7 that can skip the dry season without damage. In Africa, short maturation phases are only
8 observed in the lowlands of East Africa while long maturation phase are recorded in West and
9 Central Africa plus high-altitudes of East Africa. In Madagascar, northern species among
10 Subterminales have either very short maturation phase either very long maturation phase. This
11 indicates two opposite strategies to avoid the dry season. Three behavior have been observed
12 in Seasonally Dry Tropical Forest: (1) flowering and fruiting exclusively in in the rainy
13 season or (2) flowering in the rainy season and fruiting in the dry season or (3) flowering and
14 fruiting in the dry season (Luna-Nieves et al., 2017). The seasonal variation of irradiance
15 influenced seed development times (Zimmerman et al; 2007).

16

17 *Climate effect on traits related to plant development.*

18 Ours results shown that the climatic factors affect leaf size and mass, the diameter of green
19 internodes which is a proxy of the size of meristem (see Table 3). These features decreased
20 with increasing aridity. The size of leaves is an adaptive trait to drought (Westoby et al.,
21 2002; Wright et al., 2017). In dry climate, the leaves are small and thick with a high LMA
22 (Niinemets, 2001).

23 The leaves of *Coffea* species of humid region have short internodes and large internode
24 diameter and therefore a weak ratio of internode length to diameter. On the contrary, the
25 species of arid region have long internode and small internode diameter and thus a high ratio

1 of internode length-to-diameter. Our results are in line with Corner's rules on the leaf/stem
2 size: the species with a large leaves have a thick stem and large fruits and weak branching
3 while the species with small leaves have a thin stem and small fruits and high branching
4 (Corner, 1949). In wild *Coffea*, our results show that the species with large leaves have large
5 green internode diameter, proxy of the size of apical meristem.

6

7 A positive relationship was found between the leaf size and the internode diameter. Leaves
8 intercept light and acquire carbon, while stems transport water with nutrients and
9 mechanically support the leaves, thus leaf and stem traits are expected to be highly
10 coordinated biomechanically and physiologically (Westoby et al., 2002).

11

12 The climatic factors influence the value of ratio between internode mass and leaf mass which
13 are higher for species growing in intermediate and arid regions (see table 3). Thus, these
14 species allocate more to the stem than to the leaves. In arid environment, the species with
15 small leaves are favored because they have a high LMA and thus a better photosynthetic
16 capacity (Westoby et al., 2002). The mechanical cost is the lowest for the species with small
17 leaves (Givnish 1987). The small leaves are dense and thick (Niinemets 2001) with a low
18 transpiration (Westoby et al., 2002).

19

20 *Phylogenetic signal*

21 The phylogenetical signal of ratio of internode mass to leaf mass was highly significant (see
22 fig. 7). This ratio illustrates the variability in stem vs leaves biomass allocation between
23 species. The phylogenetic signal of the leaf dry matter content (LDMC) is significant. The
24 LDMC is an indicator of the use of plant resources, a trade-off between a rapid assimilation
25 and growth and an efficient conservation of resources within well-protected tissues (Vaieretti

1 et al., 2007). The strong conservatism of LDMC is known (Purschke et al., 2013; Kazakou et
2 al., 2014; Fort et al., 2015; Poorter et al., 2018). The stomatal length and stomata density have
3 a significant phylogenetic signal. In the *Coffea* genus, generally, the smaller genome species
4 have smaller leaves with larger stomata and grows on the dry environment while the larger
5 genome species from humid habitats have longer leaves with a higher stomata density
6 (Razafinarivo et al., 2013).

7 LMA reflects the cost of leaf construction, the light interception and the plant fitness with a
8 low phylogenetic signal (Flores et al., 2014). LMA is linked to leaf lifespan and to
9 photosynthetic capacity per mass unit (A_m). The species with low LMA have generally fast
10 growth rate (Flores et al., 2014).

11 The phylogenetical signal of leaf petiole length, leaf length, leaf area and leaf width was
12 weakly significant. The size of the leaves is mainly controlled by global climatic factors
13 (Wright et al., 2017). The phylogenetical signals of the ratio internode length-to-diameter are
14 weakly significant too. The stem size is species dependent within *Coffea*.

15 The length of the maturation phase shows a high significant phylogenetic signal. It is well
16 known that the phenology is an adaptive trait (Chuine, 2010). These results highlight the
17 assumption that the phenological sensitivity was a significant predictor of species adaptation
18 to climate change (Cleland et al., 2012).

19 .

20

21 CONCLUSIONS

22 For Madagascan wild coffees as for African species, the flowering is influenced by both the
23 climatic conditions (dry period followed by a trigger rain) and genetic parameters at the
24 species level. Phenology parameters (number of days for blossoming after the trigger rain and

1 maturation phase length) did not correlate with natural habitat type. However, a high
2 variability of length of the maturation phase (from 59 to 360 days) with a strong phylogenetic
3 signal is noted. In drier environment, two opposite strategies to avoid the dry season are
4 observed either very short as *C. boiviniana* (59 days) or very long as *C. tsirananae* (360 days)
5 that raise questions on the adaptive meaning of this trait. Reproductive strategies involve
6 mechanisms that favor species integrity (reduced gene flow and reduced hybrids growth or
7 fertility) and species survival in climate changes. The climatic factors affect leaf size and
8 mass, the diameter of green internodes, which is a proxy of the size of meristem. The ratio
9 between the stem dry mass and leaf dry mass shows a high phylogenetic signal. To our
10 knowledge, there are few studies on such phylogenetic signal.

11

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19

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TABLES

Table 1. Coffee species examined, with population code, species name, botanical series (from Charrier 1978), origin, province, GPS coordinates, habitat class. Habitat class: 1: < 2 dry months; 2 : from 2 to 6 dry months; 3 : > 6 dry months.

ID	species name	botanical series	Origin/Locality	Province	latitude coordinates	longitude coordinates	habitat class
A964	C.dubardii Jum.	Garcinioides	Anivorano Nord	Antsiranana (North)	S12°44'	E49°09'	3
A969	C.dubardii Jum.	Garcinioides	North of Voohemar (Diégo-Suarez)	Antsiranana (North)	S13°20'	E49°57'	3
A516	C.heimii J.-F.Leroy	Garcinioides	Diégo-Suarez (Sahafary)	Antsiranana (North)	S12°35'	E49°26'30"	3
A40	C. mogenetii Dubard	Garcinioides	Montagne d'Ambre	Antsiranana (North)	S49°12'	E12°26'	3
A252	C. tetragona Jum. & H.Perrier	Garcinioides	Behangony (Est de Maromandia)	Mahajanga (Northwest)	S14°13'30"	E48°09'	3
A601	C. abbayesii J.-F.Leroy	Millotii complex	Fort-Dauphin (Isaka-Ivondro)	Toliara (Southeast)	S24°45'15"	E46°51'45"	2
A572	C. ambodirianensis Portères	Millotii complex	reserve Ambodiriana	Toamasina (East)	S18°27'08"	E48°56'36"	1
A206	C. dolichophylla J.-F.Leroy	Millotii complex	Nosy Vohimanoro), Ambodilafa	Varika Fianarantsoa (Southeast)	S20°29'	E48°10'30"	1
A208	C. farafanganensis J.-F.Leroy	Millotii complex	Farafangana (Amboangibe)	Fianarantsoa (Southeast)	S22°53'	E47°48'	1
A212	C. millotii J.-F.Leroy	Millotii complex	Vatovavy	Fianarantsoa (Southeast)	S21°24'3"	E47°56'32"	1
A219	C. millotii J.-F.Leroy	Millotii complex	Ambatovaky	Fianarantsoa (Southeast)	S21°22'48"	E47°53'06"	1

A222	<i>C. millotii</i> J.-F.Leroy	Millotii complex	Mananjary (Tolongoina)	Fianarantsoa (Southeast)	S21°31'	E47°25'	2
A721	<i>C. millotii</i> J.-F.Leroy	Millotii complex	Ampasinambo (Nosy Varika)	Fianarantsoa (Southeast)	S 20° 31' 15"	E 48°01'75"	1
A575	<i>C. richardii</i> J.-F.Leroy	Millotii complex	Fenerive-Est (Tampolo)	Toamasina (East)	S17°17'	E49°25'	1
A817	<i>C. richardii</i> J.-F.Leroy	Millotii complex	Soanierana Ivongo	Toamasina (East)	S16°45'	E49°35'	1
A227	<i>C. andrambovatis</i> J.-F.Leroy	Multiflorae	Tolongoina	Toamasina (East)	S21°46'15"	E47°57'07"	1
A310	<i>C. andrambovatis</i> J.-F.Leroy	Multiflorae	Vatovavy	Fianarantsoa (Southeast)	S21°24'3"	E47°56'32"	1
A529	<i>C. ankaranensis</i> J.-F.Leroy ex A.P.Davis & Rakotonas.	Multiflorae	Standoko (Diégo-Suarez)	Antsirana (North)	S12°45'13"	E49°08'48"	3
A808	<i>C. ankaranensis</i> J.-F.Leroy ex A.P.Davis & Rakotonas.	Multiflorae	Ankarana (Diégo-Suarez)	Antsirana (North)	S12°50'57"	E49°32'36"	3
A403	<i>C. arenesiana</i> J.-F.Leroy	Multiflorae	Moramanga (Ambodivato)	Toamasina (Centre)	S18°56'	E48°12'	2
A303	<i>C. bertrandii</i> A.Chev.	Multiflorae	Italy (Fort-Dauphin)	Toliara (Southeast)	S25°02'	E46°59'	3
A5	<i>C. bertrandii</i> A.Chev.	Multiflorae	Mahampoana (Fort-Dauphin)	Toliara (Southeast)	S 25°01'	E 46°38'	3
A956	<i>C. costei</i> sp. nov. Ined.	Multiflorae	Route côtière Sambava	Antsirana (North)	S13°45'13"	E50°0'48'	2
A570	<i>C. coursiana</i> J.-F.Leroy	Multiflorae	Tamatave (Betampona RNI n°1)	Toamasina (East)	S17°53'	E49°13'	1
A315	<i>C. leroyi</i> A.P.Davis	Multiflorae	Ifanadiana (Ambodiarafia)	Fianarantsoa (Southeast)	S21°20'	E47°45'	1

A18	C. mangoroensis Portères	Multiflorae	Ambatondrazaka		Toamasina (East)	S19°16'3" "	E48°24'09"	1
A401	C. mangoroensis Portères	Multiflorae	Mangoro (Moramanga)		Toamasina (East)	S18°55'	E48°14'	2
A402	C. mangoroensis Portères	Multiflorae	Mangoro (Moramanga)		Toamasina (East)	S18°56'	E48°13'	2
A321	C. montis-sacri A.P.Davis	Multiflorae	Vatovavy		Fianarantsoa (Southeast)	S21°24'35" 5"	E47°56'32" 2"	1
A12	C. perrieri Drake Jum. & H.Perrier	Multiflorae	Fort-Dauphin (Amboasary-Atsimo)		Toliara (Southeast)	S24°50'	E46°33'55"	3
A421	C. perrieri Drake Jum. & H.Perrier	Multiflorae	Ihosy		Fianarantsoa (Southeast)	S22°33'03" 3"	E46°07'40" 0"	3
A730	C. perrieri Drake Jum. & H.Perrier	Multiflorae	Tsaratanàna		Mahajanga (Northwest)	S16°47'	E47°39'	3
A71	C. resinosa (Hook.f.) Radlk.	Multiflorae	Maroantsetra Farakaraina)	(Forêt	Toamasina	S 15°26'	E 49°44'	1
A8	C. resinosa (Hook.f.) Radlk.	Multiflorae	Nosy Varika		Fianarantsoa (Southeast)	S 20°36'26".6"	E 48°31'56".5"	1
A827	C. resinosa (Hook.f.) Radlk.	Multiflorae	Fananehana		Toamasina	S15°53'26" 6"	E49°42'19" 9"	1
A906	C. resinosa (Hook.f.) Radlk.	Multiflorae	Tanambao		Antsirana (North)	S17°43'38" 8"	E48°49'59" 9"	2
A910	C. resinosa (Hook.f.) Radlk.	Multiflorae	North Soanierana Ivongo		Toamasina (East)	S16°55'17" 7"	E49°35'07" 7"	1
A913	C. resinosa (Hook.f.) Radlk.	Multiflorae	Manompana		Toamasina (East)	S16°40'58" 8"	E49°45'17" 7"	1
A915	C. resinosa (Hook.f.) Radlk.	Multiflorae	Ivontaka		Toamasina (East)	S16°18'	E49°49'00" 0"	1
A538	C. sahafaryensis J.-F.Leroy	Multiflorae	Sahafary (Diégo-Suarez)		Antsirana (North)	S12°53'18" 8"	E49°22'	3

A978	C. sahafaryensis J.-F.Leroy	Multiflorae	North of Vohémar		Antsiranana (North)	S13°17'20"	E49°57'	3
A20	C. vianneyi J.-F.Leroy	Multiflorae	Ampasinambo (Nosy Varika)		Fianarantsoa (Southeast)	S20°32'53"	E48°07'50"	1
A946	C. vianneyi J.-F.Leroy	Multiflorae	Moramanga		Toamasina (Centre)	S18°56'	E48°15'	2
A966	C. augagneurii Dubard	Subterminales	Anivorano Nord		Antsiranana (North)	S12°43'	E49°10'	3
A973	C. boiviniana (Baill.) Drake	Subterminales	Nafokovo (Vohémar)	(Nord)	Antsiranana (North)	S13°22'13"	E49°58'12"	3
A980	C. boiviniana (Baill.) Drake	Subterminales	Vohémar		Antsiranana (North)	S13°21'	E49°52'48"	3
A535	C. bonnieri Dubard	Subterminales	Diego-Suarez (Forêt d'Ambre)	(Forêt)	Antsiranana (North)	S12°29'30"	E49°10'40"	3
A974	C. jumellei J.-F.Leroy	Subterminales	Nafokovo (Vohémar)	(North)	Antsiranana (North)	S13°22'13"	E49°58'12"	3
A957	C. pervilleana (Baill.) Drake	Subterminales	Route côtière Sambava - Diégo Suarez		Antsiranana (North)	S13°49'17"	E50°02'04"	2
A958	C. pervilleana (Baill.) Drake	Subterminales	Route côtière Sambava - Diégo Suarez		Antsiranana (North)	S13°49'17"	E50°02'04"	2
A528	C. ratsimamanga J.-F.Leroy ex A.P.Davis & Rakotonas.	Subterminales	Sandokoto (Diégo-Suarez)	(Diégo-)	Antsiranana (North)	S13°48'30"	E49°17'39"	2
A967	C. ratsimamanga J.-F.Leroy ex A.P.Davis & Rakotonas.	Subterminales	Anivorano Nord		Antsiranana (North)	S12°44'	E49°14'	3
A307	C. sakarahae J.-F.Leroy	Subterminales	Iakora (Ihosy)		Fianarantsoa (South)	S23°06'	46°39'E	3
A515	C. tsirananae J.-F.Leroy	Subterminales	Diégo-Suarez (Cap d'Ambre)	(Cap)	Antsiranana (North)	S11°58'	E49°16'	3

A308	C. vatovavyensis J.-F.Leroy	Subterminales	Vatovavy		Fianarantsoa (Southeast)	S21°24'	E47°56'	2
A830	C. vatovavyensis J.-F.Leroy	Subterminales	unknown		unknown	unknown	unknown	NA
A977	C. vohemarensis A.P.Davis & Rakotonas.	Subterminales	North of Sambava		Antsirana (North)	S14°14'	E50°8'30'	2
A574	C. homollei J.-F.Leroy	Verae	Réserve (Tamatave)	Ambodiriana	Toamasina (East)	S17°59'13"	E49°17'48"	1
A213	C. kianjavatensis J.-F.Leroy	Verae	Vatovavy		Fianarantsoa (Southeast)	S21°24'30"	E47°56'30"	1
A602	C. kianjavatensis J.-F.Leroy	Verae	Fort-Dauphin		Toliara (Southeast)	S 24°45'15"	E 46°51'45"	2
A320	C. lancifolia A.Chev.	Verae	Mananjary (Madiorano/Tolongoina)		Fianarantsoa (Southeast)	S21°31'	E47°29'	1
A405	C. lancifolia A.Chev.	Verae	Tolongoina forest)	(Kararaika)	Fianarantsoa (Southeast)	S22°57'02"	E47°22'56"	2
A571	C. lancifolia A.Chev.	Verae	Reserve	Ambodiriana	Toamasina (East)	S18°27'08"	E48°58'36"	1

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1 **Table 2:** Physical parameters of habitats corresponding to habitat class of species 1 to 3 as
 2 defined by the number of dry months.

Habitat class	Number of dry months	Annual precipitation in mm	Temperature of the coldest month in °C	Temperature of the warmest month in °C	Annual evapotranspiration in mm	potential Aridity index in mm
1	0 - 1	2051 to 3360	11.6 to 19.9	26.3 to 30.9	945 to 1289	1081 to 2142
2	2 - 6	1260 to 2265	10.6 to 20.7	23.1 to 30.9	814 to 1370	175 to 1111
3	>6	552 to 1829	10.2 to 22.8	28 to 33.1	950 to 1627	-691 to 379

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Table 3. Mean comparison between the three bioclimatic subsets of species defined on the number of dry months in the natural habitat of studied populations/species. Mean for each trait according to the number of dry month in the natural habitat of Coffee populations. Difference in means between subsets was tested with ANOVA. P-values (***<0.001, **<0.01, *<0.05) inferior to 0.05 are considered as evidence of trait divergence. LDMC: leaf dry mass content; LMA: leaf mass per area.

Habitat class	1	2	3	p value ANOVA
Leaf length (mm)***	113.05185	76.3	68.15909	0.000185
Leaf width (mm)***	48.34444	35.02857	28.92727	2.16E-05
Leaf area (mm ²)***	6150.185	3156.571	2299.262	0.00012
Two leaves mass (g)**	1.465	0.453	0.5341176	0.00925
Internode diameter perpendicular to leaves, mm)**	3.720833	2.293	2.195625	0.00454
Maturation phase length (days)**	104.6296	105.0714	178.3182	0.00308
Ratio internode mass /2 leaves mass (g)*	0.1275	0.259	0.2175	0.0487
Ratio Length of internode/internode diameter parallel to leaves*	12.78	24.117	21.7975	0.039
Number of flowers per inflorescence*	4.403704	2.8	3.177273	0.0123
Seed thickness (mm)*	3.885926	4.053077	4.673	0.0422
Leaf shape	2.362	2.17	2.588	0.412
Internode diameter (parallel to leaves, IDP, mm)	2.058333	1.587	1.636875	0.123
Petiole length	8.12	6.45	6.34	0.151
length internodes (LI, mm)	23.16667	32.1	33.35	0.056
Internode mass (IM,g)	0.2143	0.0878	0.101	0.121
Fruits length (mm)	15.86148	14.69077	15.5035	0.795
Number of days from trigger rain to Flowering	7.648148	7.321429	8.475	0.13
Fruits thickness (mm)	12.69296	11.46154	11.442	0.445
Fruit shape index	1.225926	1.169231	1.2535	0.707
Fruit volume index (mm ³)	4836.13	3041.622	2910.932	0.212
Pedicle length (mm)	3.498889	3.811538	4.293	0.108
Disk diameter (mm)	3.391111	3.181667	2.784444	0.3
Seed length (mm)	9.183704	8.683846	10.3285	0.237
Seed width (mm)	5.692593	5.041538	6.57	0.228
Seed volume (mm ³)	295.5733	219.8423	352.434	0.549
100 seed weight (g)	9.677778	8.8175	14.291333	0.138
LMA mean (g/m ²)	175.7083	137.78	202.3235	0.235
LDMC (mg g ⁻¹)	357.1	344.4143	382.1583	0.388
Mean stomata density	203.5238	152.2308	171.3182	0.0797
Stomata length (μ)	8.12	8.378923	8.225682	0.845

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FIGURE CAPTIONS

Figure 1: Phenotypic relationships among Madagascan *Coffea* species. NJ tree based on Dice's dissimilarity indices calculated from 107 variables encoded presence-absence for 364 individuals, 200 bootstraps using DARwin v5 (Perrier and Jacquemoud-Collet, 2006). Only bootstraps superior to 80% are shown. The botanical series to which each species belongs to is given in yellow for Garcinioides, red for Millotii complex, blue for Multiflorae, pink for Subterminales and green for Vereae.

Figure 2: Phenotypic tendencies. Standardized trait values in columns for each species following their phylogenetic relationships based on Hamon et al. (2017), with large black squares indicating the highest values. The serie Millotii complex is colored in red and the species with higher length maturation phase are in yellow color. The leaf-related and vegetative traits are in red, phenological traits and inflorescence trait are in yellow, fruit and seed traits are in blue, functional leaf trait are in green and stomata traits are in purple.

Figure 3: Internodes, fruits and seeds of *C. richardii* (A); Fruit of *C. tsirananae* (B); leaf of *C. heimii* (C).

Figure 4: A: Variation of the time between the triggering rain and blossoming according to the number of dry months, 0-1 for S1 or "Habitat class 1", 2-6 for S2 or "Habitat class 2" and >6 for S3 or "habitat class 3" for each population and B: Dates of blossoming according to the botanical series of each population.

Figure 5: A: Variation of the length of the maturation phase (in days) according to the length of the dry season (in number of dry months); B: Dates for ripen fruits according to the botanical series of the populations. GAR for Garcinioides, MIL for Millotii complex, MUL for Multiflorae, SUB for Subterminales and VER for Veare.

Figure 6: Reproductive strategies of Madagascan wild coffee trees. Diagram showing the flowering (blue colors) and fructification period (red colors) and germination period (green color) according to the length of the rainy/dry seasons along the year. The length of the rainy season is indicated by clouds. The number of dry months of dry season is indicated by sunshine.

Figure 7: Phylogenetic signal in coffee trees traits using Blomberg's K parameter. Indication of strong phylogenetic effect can be seen for a trait when K is close to 1 (black dashed line) and higher than the lowest threshold value of 0,52 (grey dashed line). Its significance is attested when traits are followed by an asterisk (p-value < 0.05 and consistent with Pagel's λ significant p-value <0.05) or two asterisk when When K value close to 1. The leaf-related and vegetative traits are in red, phenological traits in yellow, fruit and seed traits in blue, functional leaf trait in green and stomata traits in purple.

