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ARTICLE

Fitness consequences of hybridization in a predominantly selfing species: insights into the role of dominance and epistatic incompatibilities

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Studying the consequences of hybridization on plant performance is insightful to understand the adaptive potential of populations, notably at local scales. Due to reduced effective recombination, predominantly selfing species are organized in highly homozygous multi-locus-genotypes (or lines) that accumulate genetic differentiation both among- and within-populations. This high level of homozygosity facilitates the dissection of the genetic basis of hybrid performance in highly selfing species, which gives insights into the mechanisms of reproductive isolation between lines. Here, we explored the fitness consequences of hybridization events between natural inbred lines of the predominantly selfing species *Medicago truncatula*, at both within- and among-populations scales. We found that hybridization has opposite effects pending on studied fitness proxies, with dry mass showing heterosis, and seed production showing outbreeding depression. Although we found significant patterns of heterosis and outbreeding depression, they did not differ significantly for within- compared to among-population crosses. Family-based analyses allowed us to determine that hybrid differentiation was mostly due to dominance and epistasis. Dominance and/or dominant epistatic interactions increased dry mass, while decreasing seed production, and recessive epistatic interactions mostly had a positive effect on both fitness proxies. Our results illustrate how genetic incompatibilities can accumulate at a very local scale among multi-locus-genotypes, and how non-additive genetic effects contribute to heterosis and outbreeding depression.

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INTRODUCTION

The evolutionary forces underlying divergence between populations, in particular the relative strength of genetic drift compared to natural selection, can be revealed by studying the consequences of hybridization (Lynch 1991; Demuth and Wade 2005; Fitzpatrick 2008). Strong genetic drift (e.g., due to demographic events or mating systems, Barrett et al. 2014) increases the probability of fixation of deleterious mutations (Kimura et al. 1963). Hybridization between populations that have accumulated different recessive or partially recessive deleterious mutations is therefore expected to lead to heterosis, i.e., an increase in fitness in F_1 hybrids compared to the average fitness of their parents (Crow 1948; Lynch 1991; Glémin 2003). However, if selection is much stronger than drift, divergent populations are expected to accumulate genetic incompatibilities and hybridization may lead to a loss of fitness in F_1 and/or subsequent generations (in general the F_2 generation), called outbreeding depression (Lynch 1991). Several nonexclusive hypotheses have been proposed to explain the genetic architecture of outbreeding depression, the most prominent of which are: chromosomal rearrangement, which leads to the production of aneuploid gametes in heterozygotes (Lande 1985; Charlesworth 1992; Kirkpatrick and Barton 2006); under-dominance, which leads to lower fitness for heterozygotes compared to the homozygote genotypes (Schierup and Christiansen 1996); and negative epistatic interactions among divergent alleles that are brought together for the first time in hybrids (Dobzhansky 1937; Lynch 1991; Demuth and Wade 2005).

Predominantly selfing species are of particular interest when studying the phenotypic and fitness consequences of hybridization. Indeed, by decreasing the effective population size, selfing increases the strength of genetic drift (Glémin and Ronfort 2013), and thereby the fixation probability of deleterious mutations (Kimura et al. 1963). By increasing homozygosity and reducing the effective recombination rate, self-fertilization also increases the probability of fixation of under-dominant loci, chromosomal rearrangements (Charlesworth 1992), and negative epistatic interactions (Coyne and Orr 2004), both within and between populations (Wright et al. 2013). Finally, by organizing populations in a mosaic of repeated fully homozygous multi-locus genotypes (thereafter MLGs; Siol et al. 2008; Volis et al. 2010; Jullien et al. 2019), self-fertilization greatly simplifies the dissection of the genetic architecture of hybrid fitness. Indeed, hybridization between MLGs produces F_1 hybrids that are fully heterozygous at all sites where the parents differ, and the heterozygosity is divided by two at each subsequent generation of selfing, providing the opportunity to test the relative role of dominance and of different forms of epistasis on hybrids' performance (Demuth and Wade 2005; Fitzpatrick 2008a, see also Supplementary Methods S1).

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Outbreeding depression and heterosis have been widely documented in predominantly selfing species (Rhode and Cruzan 2005; Johansen-Morris and Latta 2006; Dolgin et al. 2007; Volis et al. 2010; Gimond et al. 2013; Oakley et al. 2015). Under predominant selfing, explanations for heterosis often involve the masking of recessive or nearly recessive deleterious mutations that can become fixed in large numbers by genetic drift within natural populations, while negative epistatic interactions are generally thought to be the main cause of outbreeding depression (Rhode and Cruzan 2005; Johansen-Morris and Latta 2006; Dolgin et al. 2007). In most cases, however, these studies lack a proper testing of the mechanisms involved, and the methods available to detect genetic interactions, such as joint scaling analyses, are rarely applied despite their usefulness and ease of application in selfing species. To our knowledge, only a single study has analyzed the underlying mechanisms of the genetic consequences of among-population crosses. By crossing multi-locus genotypes from Swedish and Italian populations, Oakley et al. (2015) found that outbreeding depression and heterosis in *Arabidopsis thaliana* was caused by dominance, while epistasis tended to increase the fitness of hybrids (Oakley et al. 2015).

Little is known about the scales at which heterosis and outbreeding depression occur in highly inbred species. Notably, due to reduced effective recombination rates, inbred lines found within a population may be as likely as geographically isolated lines to fix genetic incompatibilities among themselves. Nevertheless, most studies focus on the performance of crosses among-populations and only few studies have tried to investigate the effect of local genetic structure. Some studies found negative inbreeding depression within populations (Husband and Schemske 1996; Volis et al. 2010; Winn et al. 2011), which means that inbred progenies were fitter than outbred ones. This suggests that genetic incompatibilities between MLGs can reach fixation even at a local spatial scale. On the other hand, Gimond et al. (2013) found that crosses among MLGs sampled within the same population were less deleterious than crosses among MLGs collected in different populations. Furthering our knowledge on what happens at the population scale is important as it could notably explain why transitions from selfing to outcrossing are rare (Harkness et al. 2019) and give insight into the short-term evolutionary potential of selfing populations (Clo et al. 2020).

In this paper, we investigate the effect of hybridization between naturally occurring inbred lines from the predominantly selfing species *Medicago truncatula*. We aim at (1) quantifying the occurrence and magnitude of heterosis and outbreeding depression at both within- and among-populations scales, (2) comparing hybrid performances between the two spatial scales and (3) deepening our understanding of the mechanisms leading to heterosis and outbreeding depression by dissecting their genetic architecture using family-based analyses.

MATERIALS AND METHODS

Model species and plant material

Medicago truncatula (*Fabaceae*) is an annual, diploid plant species commonly found in the Mediterranean basin. It reproduces predominantly through self-fertilization (outcrossing rate between 1 and 7%, Jullien et al. 2019). Here, we focussed on five natural populations studied by Jullien et al. (2019): three populations collected in Corsica (CO1, CO2, and CO3), one in the south of France (FR1) and one in the south of Spain (SPA-1). Jullien et al. (2019) analyzed the genetic structure of these five populations using 20 microsatellite markers and reported that each population is dominated (sometimes over 50% of the population) by one or a few multilocus genotypes (MLGs) coexisting with several rare genotypes. For each population, we chose the two most frequent MLGs, except for the CO3 population, for which four MLGs were sampled, adding up to a total of 12 parental lines. Further details about the method used to estimate the frequency of the different MLGs can be found in Jullien et al. (2019). The genetic differentiation among these MLGs was measured using the 20 microsatellite markers, and was defined as the number of alleles that differ between two MLGs, divided by the total number of alleles without missing data. It varied between 12 and 86% within-populations and between 57 and 100% among-populations (see Table S1 for details).

Crosses

We conducted controlled crosses using the 12 MLGs originating from the five populations selected. We performed all the possible within-population crosses using the FR1, SPA1, CO2, and CO3 populations (one cross for the populations in which two MLGs were sampled, and six for the CO3 population). Due to a technical error in the crossing design, we had to remove the within-population crosses for population CO1, adding up to a total of 9 within-population crosses. For among-populations crosses, we selected the most frequent MLG within each population and the two most frequent MLGs for the CO3 population. With these six MLGs, all the possible among-populations crosses were performed, leading to a total of 14 among-populations crosses. These 23 crosses were performed in spring between 2013 and 2015.

Twenty plants for each of the 12 MLGs were grown in the greenhouse in order to have a sufficiently large number of pollen donors and mother plants, and to minimize any potential environmental maternal effect. All crosses were carried out using each MLG as a mother or pollen donor. Flower buds were sampled before the flowers opened. The flowers used as pollen donors were dissected by removing sepals and petals. For flowers used as pollen receivers, the immature anthers were carefully removed under a binocular scope and pollen from a donor was manually deposited on the stigma. The stems were then wrapped with cotton wool and placed in a tube with some water in order to maintain a moist environment and favor fertilization. Each hand-fertilized flower was then labelled. Once a fruit appeared, it indicated that the fertilization was successful and the tube was removed. Pods were left on the mother plants until fully ripe. Each cross supplied us with between 0 and 9 seeds (on average 5.7), from which one was randomly selected to include in the experiment. Several crosses were performed with each plant and between 1 and 10 seeds from each mother plant (respectively between 1 and 17 for pollen donors) were included in the experiment described below.

In 2016, a subset of seeds from the F_1 families were germinated. We randomly selected up to ten families for each cross (on average 8.8 because fewer crosses were successful for some families), adding up to a total of 219 F_1 seeds. Seedlings were grown in an insect-proof greenhouse and self-fertilized to produce F_2 plants. Leaves from mother plants used to generate the F_1 were sampled for DNA extraction in order to confirm that the cross was successful given the parental MLGs, using four microsatellite loci (B12F1, MTIC59, TA34, FMT 11, a subset of the 20 microsatellite loci used by Jullien et al. 2019). The protocol for extractions and microsatellite genotyping was the same as detailed in Jullien et al. (2019).

Greenhouse experiment

Parental MLGs were used as a control for the effect of hybridization. The seeds were obtained from controlled crosses within MLG (to minimize the experimental effect). Because we were missing a few seeds, we completed the sample with seeds produced through autonomous selfing of the mother plants. In 2017, seeds were scarified to ease germination and transferred to Petri dishes with water at room temperature for 6 h, before being placed in a stratification chamber at 5 °C for 5 days.

After stratification, an average of eight replicates from each parental MLG (hereafter noted "*P*"), 16 replicates from each F_1 cross and 64 replicates from each F_2 cross (all obtained from different flowers, as detailed above) were transferred to the greenhouse. The greenhouse is composed of eight blocks, and for each family, all the individuals of a generation were randomly placed within the eight blocks, such that each block contained one replicate for P, two for F_1 and eight replicates for F_2. Seeds that failed to germinate were replaced to include 2000 plants in total in the experiment. The germinated seeds were transplanted into 1 litre pots, filled with a substrate made of 75% sand and 25% potting soil. Throughout the entire life cycle, the plants were watered by soaking 5 min once a week. No light or temperature controls were imposed: day length varied from 9 h.day^{-1} to 15 h.day^{-1} in the region of Montpellier from November 2017 to June 2018, while recorded temperatures in the greenhouse varied on average between 4 and 13 °C at night, and between 20 and 30 °C during the day, over the same period.

All the plants that survived transplantation from Petri dishes to the greenhouse reached the flowering stage. The experiment was stopped when all reproductive plants had become senescent, in June 2018. A plant was categorized as senescent when the flower and fruit productions stopped, and that all the fruits were mature (fruits turn from green to brown at maturity).

Traits and fitness measurements

When all the plants had become senescent, we sampled all the fruits produced, and then we removed plants from the pots. The biomass was dried in an oven and weighed to estimate the dry mass (g). The fruits produced were counted and threshed manually. During this step, some seeds were broken, we therefore counted the number and the total weight of unbroken seeds and measured the total weight of broken seeds. These measures were used to estimate the number of broken seeds by dividing the total weight of broken seeds by the mean weight of an unbroken seed. Finally, we summed the number of unbroken seeds and the estimated number of broken seeds to estimate the total number of seeds produced by a plant over its entire lifespan. The total number of seeds was considered as a proxy of fitness.

Statistical analyses

Preliminary analyses. We first computed the survival probability of the plants as a function of the generation of individuals (P, F_1 or F_2). As the survival rates were high and that we cannot distinguish natural death and death from manipulation during transplantation, we did not perform statistical tests on this metric. We then investigated if the flowering time of individuals was correlated with either the dry mass or the seed production, by using linear models. Finally, we tested whether the dry mass and the number of seeds produced by individuals were correlated, using a linear model.

Hybrid performance at different spatial scales. For the two traits studied and for each family (one family includes the two parental lines and the associated $F1$ and $F2$ hybrids), we computed the relative performance (*RP*) of hybrids compared to the mid-parent values as

$$RP = \frac{\overline{W_H} - \overline{W_P}}{\overline{W_P}},$$

where $\overline{W_H}$ is the mean value of the trait of either the F_1 or F_2 progeny of a family, and $\overline{W_P}$ is the mid-parent value. We computed the 95% confidence intervals around the mean values by performing 10,000 bootstraps on hybrids (F_1 or F_2). A negative *RP* indicates outbreeding depression, and a positive *RP* indicates heterosis. We tested whether the mean *RP* of F_1 or F_2 differed significantly from zero within each family, by performing univariate *t*-tests. Due to the large number of tests, we corrected the significance threshold using the Bonferroni correction: the null hypothesis $RP = 0$ was rejected for $p < 0.05/92$ (23 families, for two generations and two traits) $= 5.4 \times 10^{-4}$.

Then, we tested if the average *RP* among all families ($n = 23$) differed significantly between the within- ($n = 9$ families) and among-populations ($n = 14$ families) scales, and if the genetic differentiation among inbred lines was correlated to the *RP* of hybrids, for the two generations of hybrids, and for the two focal traits, using *F*-tests. For these analyses, we used all *RP* values, whether they differed significantly from zero or not (Gimond et al. 2013).

Genetic architecture of hybrid performances. Following how the F_2 mean value differs from the mid-parent value, compared to the F_1 mean value, provides insights into the contribution of dominance and epistasis to outbreeding depression and heterosis. Here, we used the theoretical framework introduced by Fitzpatrick (2008) to incorporate epistatic interactions into line-cross quantitative genetics analyses (Demuth and Wade 2005). Indeed, classical models only consider the effect of dominant-by-dominant epistatic interactions on hybrid performance (Johansen-Morris and Latta 2006; Oakley et al. 2015), ignoring that the other forms of epistasis (additive-by-additive, additive-by-dominant and dominant-by-additive) are expected to contribute in the $F2$ generation (Fitzpatrick 2008). Here, we also considered recessive epistatic interactions, where the effect on the trait is small if both loci are heterozygous (dominant-by-dominant epistasis), and is large when at least one locus is homozygous (Fig. 1B in Fitzpatrick 2008, additive-by-dominant and additive-by-additive epistasis). Indeed, and as described in Supplementary methods S1, fully dominant epistatic interactions, where the effect on the trait is the same whether loci are heterozygous or homozygous (Fig. 1A in Fitzpatrick 2008), cannot be distinguished from dominance effects, because when epistatic interactions are dominant, epistatic effects decrease by the same order of magnitude as dominance effects between the F_1 and the F_2 generations. We tested if deviations of F_1 and F_2 offspring from mid-parent values were due to dominance (which includes single locus dominance effects and dominant

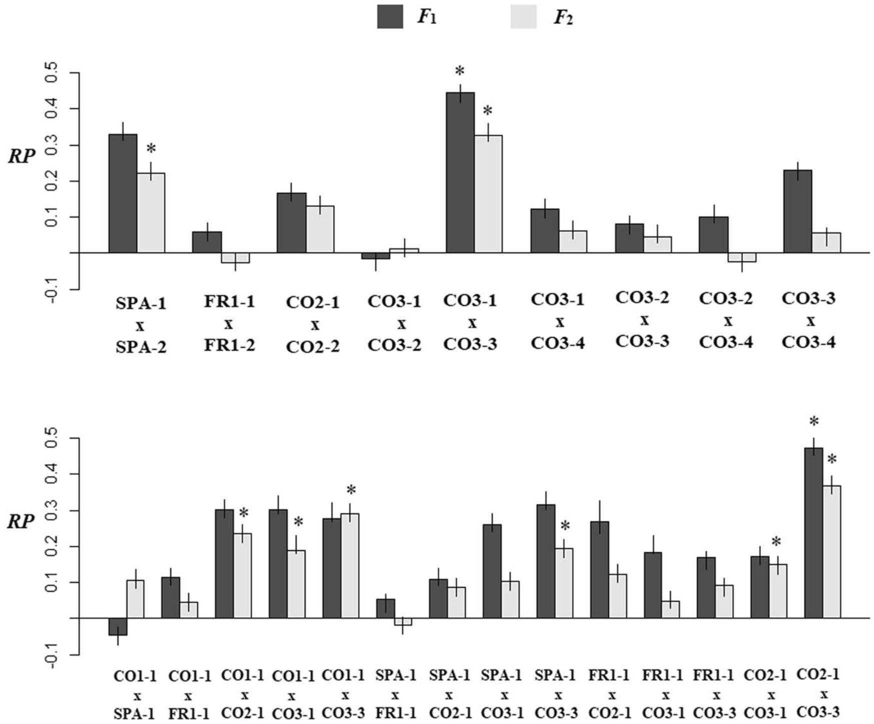

Fig. 1 Relative performance (*RP*) of F_1 (dark gray) and F_2 (light gray) hybrids for the vegetative dry mass (g), for each family, at the within-population (top panel) and among-population scale (bottom panel). Error bars stand for 95% confidence intervals, and asterisks indicate significant *RP* values with $p < 5.4 \times 10^{-4}$ (significance threshold corrected for multiple testing, see the Material and Methods section for details).

Table 1. Contribution of different genetic effects (I_A for additivity, I_D for dominance and I_E for epistasis) to plant trait value for each generation of cross.

Generation	I_A	I_D	I_E
Parental line 1	1	0	0
Parental line 2	−1	0	0
F_1	0	1	0
F_2	0	0.5	1

Additive effects differentiate parental lines compared to mid-parent value. Dominance applies in hybrids, F_1 hybrids are fully heterozygotes for differentiated loci, and heterozygosity is divided by two in F_2. Epistasis is assumed to be recessive, such that it has negligible effects when loci are in the heterozygote state, and is expressed only under homozygosity.

epistatic interactions), recessive epistatic interactions, or a combination of both, by decomposing the phenotypic variance of the studied traits into additive, dominance and epistatic components as follows: let $Z_{i,j}$ be the observed phenotypes of the *j*th individual of the *i*th family and

$$Z_{i,j,k,l} = \mu_i + I_{A,j}.A + I_{D,j}.D + I_{E,j}.E + Block_k + \varepsilon_{i,j,k}$$

where μ_i is the mean of the *i*th family under pure additivity (equal to the mid-parent value), *A* is the deviation due to additivity, *D* is the deviation due to dominance and dominant epistatic interactions, and *E* is the deviation due to recessive epistatic interactions. The contribution of each of these three fixed effects (*I* terms) depends on the generation of cross of the *j*th individual (parent, F_1 or F_2), as detailed in Table 1. $Block_k$ is the random effect corresponding to the block in which the *j*th individual was growing in the greenhouse, and $\varepsilon_{i,j,k}$ is the residual error. We compared the simplest model, limited to the mean, the additive effect and the block effect (model 1, as in Oakley et al. 2015), with models including one additional parameter, *D* (model 2) or *E* (model 3), or two additional parameters, $D + E$ (model 4).

For model selection, we used the Akaike Information Criterion (AIC, Burnham and Anderson 2002). Models that differed by less than two points of AIC from the model with the lowest AIC ($\Delta AIC < 2$), and with the same number of parameters, were considered as strongly supported by the data. Models with AIC differences lower than 2 compared to the model with the lowest AIC, but that differed from it by a single or two variables, were considered as not supported, because the additional parameters do not improve the likelihood enough to compensate for the cost of a more complex model (Burnham and Anderson 2002 p. 131). We limited the analysis to families in which at least one generation of hybrids differed significantly from the mid-parent value (eight families for dry mass and nine families for number of seeds), as we would not have the statistical power to detect a deviation from the additive model in the other families.

RESULTS

Preliminary results

From the initial 2000 plants, 1788 individuals survived the transplantation events, and all these individuals reached the flowering stage. The survival rates were of 82.7%, 87.5%, and 90.1% respectively for the parental, F_1 and F_2 generations. We found no correlation between flowering time and dry mass ($F_{1,55} = 0.28$, $p = 0.63$) or seed number ($F_{1,55} = 2.30$, $p = 0.09$), which confirmed that the experiment lasted long enough to allow late flowering individuals to end their reproductive cycle. The dry mass and the seed production of plants were negatively correlated ($F_{1,1782} = 625.50$, $p < 10^{-15}$, $R^2 = 0.26$).

Heterosis and outbreeding depression in M. truncatula crosses

The untransformed means of parental and hybrid lines for each family and both traits are given in supplementary material (Figs. S2 and S3). In several families, hybrids (F_1 or F_2) showed mean values outside the range of values for parental lines (14 families for the dry mass, and 12 for the seed production, Figs. S2 and S3).

For vegetative dry mass, we generally found positive *RP* values in both the F_1 and the F_2 generations (Fig. 1), with the increase of fitness being generally smaller in F_2 than in F_1 (Fig. 1). More rarely, the *RP* values were negative in F_1 (in the within-population cross CO3-1 × CO3-2, and in the among-population cross CO1-1 × SPA-1) or in F_2 (in the within-population crosses FR1-1 × FR1-2 and CO3-2 × CO3-4, and in the among-population cross SPA-1 × FR1-1). Due to the correction for multiple tests, among the 23 studied families, hybrids were significantly different from the mean of parental lines in eight families for F_2 hybrids and two families for F_1 hybrids (Table S2).

For the number of seeds produced per individual, we observed a general pattern of outbreeding depression (i.e., negative values of *RP*) in hybrids (Fig. 2). Crosses involving the CO3 population were an exception, with heterogeneous patterns of hybrid performance, including both heterosis (i.e., positive values of *RP*) and outbreeding depression (Fig. 2). The reduction of fitness was generally stronger in the F_1 generation, but in some cases the F_2 generation showed a stronger loss of fitness (in the within-population cross CO2-1 × CO2-2, and in the among-population crosses CO1-1 × SPA-1 and SPA-1 × CO2-1). Hybrids were significantly different from the mid-parent value in nine families out of 23 (three for F_1 hybrids, eight for F_2 hybrids, Table S2).

Effect of the spatial scale on hybrid performance

For both traits, we did not detect any effect of the spatial scale of the crosses (i.e., within vs between populations crosses, Figs. 1 and 2, Table 2), nor of the generation of cross (F_1 vs F_2, Table 2) on the RP of hybrids. In agreement with this, the *RP* values were independent of the genetic differentiation among parental lines for both dry mass and seed production for both generation of crosses (Table 2).

For the dry mass, the mean *RP* was equal to 0.195 (s.d. ±0.134) in F_1 hybrid and to 0.122 (±0.110) in the following generation (F_2). *RP* values in F_1 were on average equal to 0.169 (±0.144) at the within-population scale and to 0.211 (±0.131) at the among-population scale. In the F_2 generation, the mean *RP* was 0.089 (±0.118) at the within-population scale and 0.144 (±0.104) at the among-population scale.

For the number of seeds, the mean *RP* was −0.181 (±0.227) in F_1, and rose to −0.099 (±0.204) in F_2. The mean *RP* in F_1 was equal to −0.127 (±0.293) at the within-population scale and −0.217 (±0.177) at the among-population scale, and was equal to −0.046 (±0.271) at the within- population scale and −0.134 (±0.148) at the among-population scale in *F*2 hybrids.

Genetic architecture of heterosis and outbreeding depression

Family-based analyses on both traits gave very similar and concordant results (Table 3). For vegetative dry mass, we rejected the fully additive model for six families out of the eight exhibiting *RP* values significantly different from zero (Table 3). In these six families, dominance and epistasis always had a positive effect on dry mass (Table 3). For seed production, we rejected the fully additive model for seven families out of the nine exhibiting *RP* values significantly different from zero (Table 3). For this trait, dominance always decreased the number of seeds produced by an individual, while epistasis increased it, except for one cross (CO1-1 × SPA-1, Table 3).

For the five families for which we observed a significant *RP* for both dry mass and seed number, the genetic effects involved were highly similar. For three families, hybrid differentiations from mid-parent were due to dominance (SPA-1 × SPA-2, CO1-1 × CO2-1, and CO2-1 × CO3-3 families, Table 3). The CO3-1 × CO3-3 family also showed an increase in dry mass due to dominance and a reduction of the number of seeds due to a negative effect of dominance, but there was also a significant effect of epistasis increasing the seed number (Table 3). For the SPA-1 × CO3-3 family, all our models had similar AICs (Table 3), which could suggest that hybrid performance is controlled by a more complex model than those we could test.

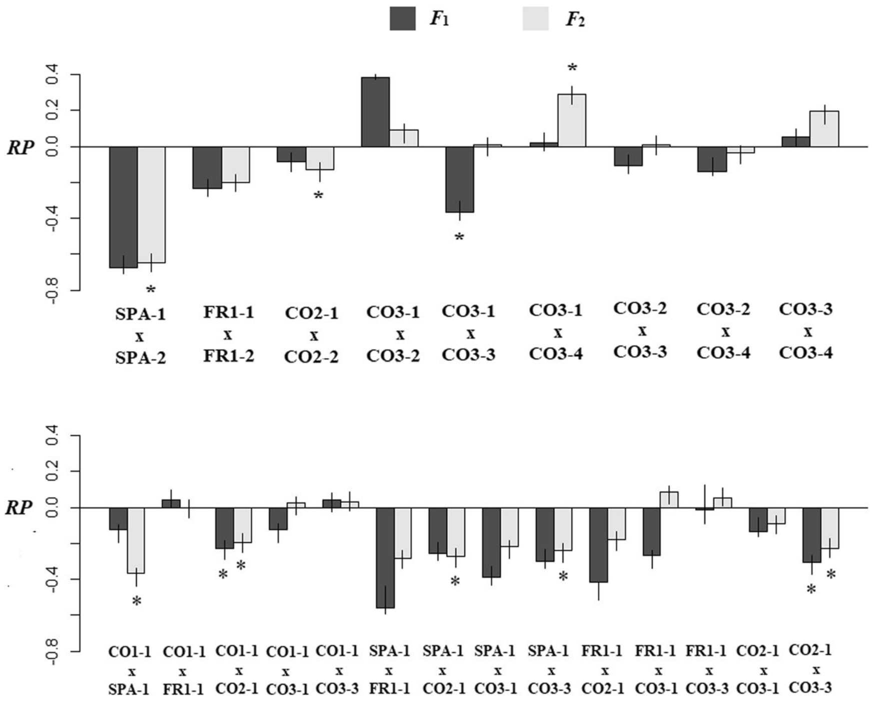

Fig. 2 Relative performance (*RP*) of F_1 (dark gray) and F_2 (light gray) hybrids for the number of seeds, for each family, at the within-population (top panel) and among-population scale (bottom panel). Error bars stand for 95% confidence intervals, and asterisks indicate significant *RP* values with $p < 5.4 \times 10^{-4}$ (significance threshold corrected for multiple testing, see the Material and Methods section for details).

Table 2. Effect of the spatial scale (within- or among-populations crosses) and of the generation of cross (F_1 or F_2) on the average relative performance (RP) over the 23 families.

Trait	Effect	df	*F*	*P*
Dry mass	Generation	1	3.794	0.059
	Spatial scale	1	1.633	0.209
	Spatial scale × Generation	1	0.026	0.872
	Genetic distance × Generation	2	0.088	0.916
Seed number	Generation	1	1.611	0.212
	Spatial scale	1	1.780	0.190
	Spatial scale × Generation	1	0.001	0.987
	Genetic distance × Generation	2	0.331	0.720

For most of the other families (for which the difference between mid-parent and hybrids was not significant), we detected that, on average, the *RP* value is divided by 2 between the F_1 generation and the F_2 generation (Fig. S4), which suggests that dominance and/or dominant epistatic interactions are also the most plausible mechanisms underlying hybrid performance in these families (Supplementary Methods S1).

DISCUSSION

In this study, we have shown that the consequences of hybridization events between multi-locus genotypes of *Medicago truncatula* are variable depending on the trait considered. Hybridization increased on average the dry mass of plants while decreasing on average seed production. Mean values of heterosis and outbreeding depression remained moderate, with deviations of around 20% on average from the mid-parent value, in agreement with previous studies made in predominantly selfing species (Dolgin et al. 2007; Volis et al. 2010; Gimond et al. 2013; Oakley et al. 2015). Yet, in contradiction with a previous study (Gimond et al. 2013), we found no significant effect of the spatial scale of crosses, as heterosis and outbreeding depression levels did not differ significantly between crosses performed at the within and among-populations levels. We dissected the genetic architecture of hybrid performances, and we found that recessive epistatic interactions mainly had a positive effect on the two studied traits, while dominance and/or dominant epistatic interactions tended to increase plants' dry mass and decrease plants' seed production, in agreement with a similar study led in *Arabidopsis thaliana* (Oakley et al. 2015).

Hybrid performance and the genetic basis of hybrids differentiation

Our observation of frequent heterosis for dry mass and prevalent outbreeding depression for seed production could be considered as a surprising result since both traits are often considered as fitness proxies, and are generally positively correlated (Younginger et al. 2017 for a review). Yet, studies in natural and laboratory accessions of the highly selfing species *Arabidopsis thaliana* also found a negative relationship between dry mass and a fitness proxy (pollen viability in Nasrallah et al. 2000; seed production in Barth et al. 2003; fruit production Vasseur et al. 2019). In the sister species *A. lyrata*, Li et al. (2019) also found that selfing populations exhibit an increase in above- and below-ground biomass, and a slight decrease in fitness (measured as the probability of bolting) in outcrossed progeny of selfing populations. It is known that environmental factors, such as nutrient or temperature, have a key role in the transition from vegetative to reproductive stages, like in flowering probability (see Cho et al. 2017 for a review). If all our surviving plants reached flowering stage, it is possible that the ecological conditions found in the greenhouse, that are different from those in the field, might have modified trade-offs between vegetative growth and investment to reproduction.

Table 3. Results of family-based analyses performed to dissect the genetic architecture of hybrid performance.

Trait	Cross	Model 1 A	Model 2 A + D	Model 3 A + E	Model 4 A + D + E	D	E
Dry mass (g)	SPA-1 × SPA-2	198	**192**	200	193	+	
	CO3-1 × CO3-3	241	**231**	243	231	+	
	CO1-1 × CO2-1	306	**297**	305	297	+	
	CO1-1 × CO3-1	320	**312**	321	313	+	
	CO1-1 × CO3-3	277	273	274	**270**	+	+
	SPA-1 × CO3-3	**322**	321	324	323		
	CO2-1 × CO3-1	**260**	260	262	261		
	CO2-1 × CO3-3	260	**254**	262	253	+	
Number of seeds	SPA-1 × SPA-2	559	**552**	558	551	–	
	CO2-1 × CO2 -2	**260**	260	262	262		
	CO3-1 × CO3-3	925	920	921	**918**	–	+
	CO3-1 × CO3-4	932	934	**928**	930		+
	CO1-1 × SPA-1	339	341	**334**	336		–
	CO1-1 × CO2-1	306	**297**	305	297	–	
	SPA-1 × CO2-1	933	**931**	932	931	–	
	SPA-1 × CO3-1	322	321	324	323		
	CO2-1 × CO3-3	971	**967**	973	968	–	

Only families where at least one generation of hybrids is significantly different from the mid-parent value are considered. Values indicate the AIC of the different models (A = Additive, D = Dominance, E = Epistasis); the best AIC is indicated in bold (see material and method section for selection criteria). The two last columns indicate the direction of the effect of dominance (D) and epistasis (E) on hybrids performance: "+" indicates a positive effect on the trait, "–" indicates the opposite.

When hybrids differed significantly from mid-parent values, the genetic basis of the differentiation was surprisingly uniform among families. In most cases, we detected signals of dominance effects (which can be attributed to either single locus dominance effects or to dominant epistatic interactions), with a negative effect on fitness. In some cases, we found signals of recessive epistatic interactions which had most of the time a positive effect on the traits. Similar results were also found by Oakley et al. (2015) and Monnahan and Kelly (2015). This result echoes with recent theoretical findings pointing out that epistasis is not always deleterious for fitness (Dagilis et al. 2019).

The opposite effect of dominance on dry mass and seed production could suggest a different genetic basis for these two traits. Heterosis for dry mass could be due to the masking of recessive deleterious mutations fixed in the different inbred lines (Charlesworth and Willis 2009), while outbreeding depression for seed production could be due to chromosomal rearrangement, or under-dominant loci, as suggested by Oakley et al. (2015) for *A. thaliana* in a study similar to ours. The different hybrid patterns observed on these two traits could also be explained if the dominance effects and epistatic interactions have a directionality and increase the dry mass in hybrids beyond the value of the mid-parent, but result in outbreeding depression for seed production due to the negative relationship between seed production and dry mass. Such a mechanism has been demonstrated theoretically by Clo and Opedal (2021), who found that dominance relationship among alleles expressed in hybrids from crosses among MLGs can generate extreme and unfit phenotypes, leading to a reduction of fitness in F_1 hybrids.

One important limitation of our study is that we reached the limits of the family-based analyses, in that we cannot distinguish the effect of dominance and dominant epistatic interactions with our design. This prevents us from describing with precision the genetic architecture of the traits studied. Adding other generations of crosses would not necessarily help distinguishing these two effects. For example, the effect of dominance and dominant epistasis is again exactly divided by two between *F*1 and back-crossed individuals, back-crosses are therefore not useful to discriminate both effects. Using *F*3 or further generations of hybrids would not help either, as dominance and epistasis will have small effects on hybrid values for those generations. Yet, arguably, there are no reasons why recessive epistatic interactions would always have a positive effect on traits, as shown by our results, while dominant epistatic interactions would always be deleterious for fitness. This reinforces our conviction that dominance rather than dominant epistatic interactions is the cause of hybrids deviation in at least some of our families.

As a next step, quantifying the number of genes (major-effect loci or generalized effect across many loci), and their mode of action (dominance vs under-dominance vs chromosomal rearrangement vs epistatic interactions) would allow to distinguish between the different modes of outbreeding depression.

Outbreeding depression in predominantly selfing species: within vs among-populations scales

A loss of fitness following crosses between lines in predominantly selfing populations has been reported frequently at the among-population scale (Rhode and Cruzan 2005; Johansen-Morris and Latta 2006; Dolgin et al. 2007; Gimond et al. 2013; Oakley et al. 2015; Vasseur et al. 2019). Yet, selfing lineages are expected to accumulate genetic differentiation and potentially incompatibilities even within populations, since genetic exchanges between plants are rare or absent when the selfing rates are very high. In this study, we found no significant difference of RP between hybrids generated through crosses within- or among-populations. Few data comparing outbreeding depression at different spatial scales are available in the literature. In contrast with our results, Gimond et al. (2013) found that hybrids of *Caenorhabditis elegans* generated by crossing lines from the same population experience smaller outbreeding depression compared to among-populations hybrids. Another study in *C. elegans* from Dolgin et al. (2007) found that within-population hybrids perform as badly as among-populations hybrids, Rhode and Cruzan (2005) found a similar pattern in *Priquieta caroliniana*, but none of these studies included statistical tests. Such results raise the question of defining what a selfing population is. In the extreme case, we could consider each MLG as a single population that interacts with other MLGs in a metapopulation network. More studies at the within-population scale are required to conclude on this question.

Consequences for adaptation in predominantly selfing species

Self-fertilization is hypothesized to be an evolutionary dead-end (Stebbins 1957; Takebayashi and Morrell 2001; Igic and Busch 2013). A classical argument to support this hypothesis is that phylogenetic transitions from predominant selfing to outcrossing are rare (Barrett et al. 1996; Escobar et al. 2010; Harkness et al. 2019). This is because the evolution of selfing is theoretically associated with a diminution of inbreeding depression through the purging of deleterious mutations (but see Winn et al. 2011 for equivocal empirical support), which then allows evolution toward even higher selfing rates (Lande and Schemske 1985). Another argument is that selfing species are more prone to extinction (Goldberg et al. 2010), notably because selfing species are expected to harbour less genetic diversity (Charlesworth and Charlesworth 1995; Clo et al. 2019). Predominant selfing leads to an organization of populations into repeated MLGs, which can all be adapted to local conditions but by combining different sets of alleles (Lande and Porcher 2015; Abu Awad and Roze 2018). Here we showed that hybridization between those MLGs, both within and among populations, is mildly deleterious, at least for seed production. This should lead to selection against a reversion from selfing to outcrossing. The low additive variance for residual outcrossing found in a population of *M. truncatula* supports this idea (Jullien et al. 2021).

Nevertheless, rare outcrossing events could be of major importance for the adaptation of selfing populations to environmental changes. Using simulations, Clo et al. (2020) showed that adaptation in predominantly selfing populations can rely on hidden genetic diversity stored into negative associations between loci (linkage disequilibrium). Rare outcrossing events occurring between differentiated MLGs are required in order to mobilize this genetic variability. Yet, this theoretical model assumes a purely additive genetic determinism for the phenotype while the results of our experiment rather suggest that dominance and/or epistatic interactions are important within predominantly selfing populations. If hybrids between MLGs are unfit, they will be counter-selected, which will prevent the release of the hidden genetic diversity and could potentially lead to the extinction of the population due to a lack of genetic variability.

CONCLUSIONS

In this study, we have shown that dominance and epistasis can be major drivers of phenotypic and fitness consequences of hybridization events between naturally occurring inbred lines of a predominantly selfing species. In addition, we have demonstrated that hybridization consequences are as important in crosses involving MLGs from the same population and crosses involving MLGs from different populations. Our study emphasizes the limits of line-cross analyses to dissect the genetic architecture of hybrid performance, and the need to perform genomic and association genetics experiments to dissect more precisely the genetic mechanisms involved in outbreeding depression. Altogether, our results bring to light the relevance of considering dominance and epistasis as drivers of the evolution of self-fertilization and of the adaptive potential of inbred populations, and the necessity to consider them in theoretical and experimental studies at both phenotypic and fitness scales.

DATA AVAILABILITY

The complete dataset and codes have been deposited on DRYAD (https://doi.org/10.5061/dryad.5x69p8d3p).

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AUTHOR CONTRIBUTIONS

L.G. initiated the project. L.G., J.R. and J.C. collected the data. J.C. performed the analyses and wrote the first draft of the manuscript. L.G., J.R. and J.C. edited the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

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