Detecting directional epistasis and dominance from cross-line analyses in alpine populations of *Arabidopsis thaliana*

Running title: Directionality of non-additive genetic effects

Arnaud Le Rouzic [1*], Marie Roumet [2,3], Alex Widmer [3], Josselin Clo [4*]

[1] Université Paris-Saclay, CNRS, IRD, UMR Evolution, Génomes, Comportement et Ecologie, France. ORCID 0000-0002-2158-3458, arnaud.le-rouzic@universite-paris-saclay.fr

[2] CTU Bern, University of Bern, Bern, Switzerland. ORCID 0000-0001-6655-1428, marie.roumet@ctu.unibe.ch.

[3] Institute of Integrative Biology, ETH Zurich, Zürich, Switzerland. ORCID 0000-0001-8253-5137, alex.widmer@usys.ethz.ch.

[4] Univ. Lille, CNRS, UMR 8198—Evo-Eco-Paleo, Lille, France. ORCID 0000-0002-3295-9481, josselin.clo@gmail.com

*corresponding author

Data availability: Data and code are available at https://github.com/lerouzic/Epicross

Authors contributions: J.C. initiated the project. M.R and A.W. collected the data. A.L.R. developed the statistical framework and J.C. and A.L.R. analyzed the data. All authors wrote the first draft and edited the manuscript.

Conflict of interest: The authors have no conflict of interest to declare.

Abstract: The contribution of non-additive genetic effects to the evolutionary potential of populations has been a topic of theoretical and empirical interest for a long time. Yet, the empirical study of these effects in natural populations remains scarce, perhaps because measuring dominance and epistasis relies on heavy experimental line crosses. In this study, we explored the contribution of dominance and epistasis in natural alpine populations of *Arabidopsis thaliana*, for two fitness-related traits, the dry weight and the number of siliques, measured in a greenhouse. We first found that, on average, crosses between inbred lines of *A. thaliana* led to mid-parent heterosis for the dry weight, but outbreeding depression for the number of siliques. While heterosis for the dry weight was due to dominance, we found that outbreeding depression for the number of siliques could be attributed to the breakdown of epistasis. We simulated and discussed the implication of these results for the adaptive potential of the studied populations, as well as the use of line-cross analyses to detect directional non-additive genetic effects.

Keywords: Non-additive genetic effects, dominance, epistasis, quantitative genetics, self-fertilization, *Arabidopsis*.

INTRODUCTION

Non-additive genetic effects generally refer to interactions between alleles at a given locus (dominance) and at different loci (epistasis), even if other forms of non-additive effects exist (Banta & Richards, 2018). Nevertheless, one consensus derived from theoretical studies is that non-additive genetic effects only influence populations' adaptive potential if epistatic or dominant effects follow a general pattern, referred to as directional dominance or epistasis (Kelly, 1999; Carter *et al.*, 2005). When genetic effects, measured locus-by-locus, are substantial, but cancel out each other when summed up over the whole genome, the population evolves as predicted under the additive approximation (Carter *et al.*, 2005).

Epistasis has the potential to modify the short- and long-term adaptive potential of a species (Cheverud & Routman, 1995; Carter *et al.*, 2005; Hansen, 2015). Directional positive epistasis tends to amplify the effect of alleles that increase the trait value. Among the consequences of positive epistasis, (i) the phenotype associated with a genetic background accumulating alleles that increase the trait value will be larger than expected by summing up the additive effects, (ii) the genetic variance of the trait will increase when the trait mean increases, and consequently, (iii) the capacity of the population to respond to selection will also increase (Carter *et al.*, 2005). Overall, directional epistasis thus makes selection response easier in the direction of epistasis, while evolution opposite to epistasis (e.g., towards lower trait value for positive epistasis) will be slower (Le Rouzic, 2014). Among other observations, directional epistasis is a good candidate for explaining asymmetric responses to bidirectional selection, a frequent but unexpected observation (Walsh & Lynch 2018, Pélabon *et al*., 2021). Despite its interest, estimates of directional epistasis for morphological traits are currently rare and not consistent (Le Rouzic, 2014). Pavlicev *et al.* (2010) showed a negative average directionality

of epistasis for body-composition traits in *Mus musculus*, while in several plant species, epistasis tended to be positive, on average, for different floral morphological traits (Johansen-Morris & Latta, 2006; Monnahan & Kelly, 2015; Oakley *et al.*, 2015; Clo *et al.*, 2021). In contrast, epistasis on fitness and fitness-related traits has received substantially more interest. Theoretical work has indeed repeatedly pointed out that epistasis on fitness should drive many diversity-generating mechanisms, including the evolution of sex, recombination, and mutation rates (Phillips *et al.*, 2000). Yet, empirical estimates remain equivocal (Maisnier-Patin *et al.*, 2005; Kouyos *et al.*, 2007; Bakerlee *et al.*, 2022).

The directionality of dominance in quantitative genetics received less theoretical attention because its consequences on the evolutionary potential of a species are case-specific (Walsh & Lynch, 2018). Directional dominance modifies the adaptive potential of a trait (Clo *et al*., 2019, Clo & Opedal 2021, Sztepanacz *et al*., 2023), but its effect depends on inbreeding (Kelly, 1999): non-additive effects can contribute to the covariance between parents and offspring in inbred populations, which does not occur under random mating (Falconer, 1996; Lynch & Walsh, 1998). The adaptive potential of inbred populations is thus not only described by the additive variance (Cockerham & Weir, 1984; Wright & Cockerham, 1985), as directional dominance contributes to the evolvability of a trait (Clo & Opedal, 2021). Directional (negative) dominance is routinely observed in fitness traits, as shown by the ubiquity of inbreeding depression (Charlesworth & Willis, 2009), but less is known about morphological traits, for which dominance can be either positive or negative, pending on studies and traits measured (Shaw *et al.*, 1998; Kelly & Arathi, 2003; Oakley *et al.*, 2015; Clo *et al.*, 2021).

Several experimental protocols have been proposed to detect the non-additive genetics effect (see Le Rouzic, 2014 for a list of methods to detect directional epistasis). Among all,

line-cross analyses are the most popular (Demuth & Wade, 2005), probably due to the simplicity of the crossing design and associated statistics, which largely predate modern molecular biology. This method requires the plants to be grown and measured in controlled conditions (generally greenhouses) in order to minimize the environmental variance and maximize the statistical power to detect genetic effects (Walsh & Lynch, 2018)

As a model species in evolutionary biology, *Arabidopsis thaliana* is a natural choice to study the directionality of non-additive gene effects in plants. *A. thaliana* (L.) Heyhn. (Brassicaceae) is native to Eurasia and North Africa but is now widely distributed throughout the Northern hemisphere (Hoffmann, 2002). This species occurs in many mountain ranges, and has been reported along a wide altitudinal range, from sea level up to 2000 m in the central Alps (Hoffmann, 2002). Unlike close relatives, *A. thaliana* is a predominately self-fertilizing, annual species; average outcrossing rates in natural populations have been reported to vary between 0.3% and 2.5% (Abbott & Gomes, 1989; Bergelson *et al.*, 1998; Picó *et al.*, 2008).

In this study, we explored the directionality of dominance and epistasis in natural Alpine populations of *A. thaliana*, for two fitness-related traits, the dry weight and the number of siliques. We first found that, on average, crosses between inbred lines of *A. thaliana* led to heterosis for the dry weight, but outbreeding depression for the number of siliques. We found that heterosis for the dry weight could be attributed to positive directional dominance, likely due to the masking of recessive deleterious mutations segregating in the different inbred lines. For the number of siliques, however, we found that outbreeding depression was likely due to positive directional epistasis.

MATERIAL AND METHODS

Study populations

We studied six natural alpine populations located along an altitudinal gradient in the Alps in the Saas Valley (Valais, Switzerland). Focal populations (Table S1) were selected from those studied by Luo *et al.* (2015). Three populations are from low altitudes (*i.e.* altitudes ranging from 850 to 1000m) and three from close to the high-elevational range margin of the species in the Alps (*i.e.* altitudes ranging from 1792 to 2012m). Distances among populations ranged from 0.8 to 25.8 km, with average distances of 6.3 km among low-altitude and 1.9 km among high-altitude populations. For our crossing experiment, we used offspring of plants collected in 2007 that were propagated in the greenhouse for three generations by single-seed descent to standardize maternal effects.

Experimental design and measured traits

Seeds of plants originally collected in 2007 and propagated for three generations by single seed descent in a greenhouse were used as parental lines in the crossing experiment. From each of the six study populations we randomly selected four parental lines with different genotypes based on results reported in Luo *et al.* (2015). Briefly, Luo *et al.* (2015) genotyped individuals using twenty-two of the twenty-four microsatellite markers (Table S2); the populations structure was reported in their Table 1 and Table S1. In the following section, when an analysis is referring to be done at the “lines” scale, it means we compared results between parental lines (seeds propagated for three generations by single seed descents). When we are referring to analyses done at the “populations” scale, we compared results between populations (a population being a set of genetic lines sampled in the same geographic location). All parental

lines were bred into four offspring categories: (i) spontaneous self-fertilization, leading to the same parental genotype, (ii) outcrossing with pollen from another parental line from the same local population, (iii) outcrossing with a parental line from another population of the same altitude, and (iv) outcrossing with a parental line from another population of the other altitude. For each parental line, self-fertilization and the three cross types were realized on four different plants. Crosses were performed using the pollen of plants exclusively grown for pollen production. Parental lines were randomly matched without replacement such that all four parental lines of each population were included as both seed and pollen parents in all cross types (Table S3). The F2 generation was produced by the spontaneous self-fertilization of one individual from each F1 family (for an overview of the crossing design see Table S3). Crosses were realized by emasculating and manually pollinating 4 to 6 flowers per female/mother plant. To avoid uncontrolled cross-pollination between neighboring plants, unused flowers were removed, and pots were individually packed in Arabisifter floral sleeves (Lehle Seeds, Round Rock, Texas, USA). To ensure self-fertilization and limit accidental crossing events, selfing plants were also packed in floral sleeves during the flowering stage.

Performance and phenotypic variation in all F1 and F2 families were assessed in a single large greenhouse experiment conducted in spring 2014. Seeds were sown on March 4th 2014 and stratified at 4 °C in the dark for six days. Plants were then grown in a greenhouse at ETH Zurich research station Lindau-Eschikon under long-day conditions (*i.e.* 10^4 Lux light for 16 h, dark for 8 h; 22 °C/18 °C day/night temperatures). From each parental line, we grew 24 F1 and 48 F2 offspring. The F1 included six offspring derived from selfing and six offspring derived from each of the three cross types. The F2 generation encompassed six selfed offspring and 14 offspring from each of the three cross types. In total, the experiment encompassed 1728 plants in total (for details see Table S3).

Plants were grown individually in 7×7×8 cm pots randomly arranged in two greenhouse compartments, and were filled with Biouniversalerde (Oekohum GmbH, Herrenhof, Switzerland), an all-purpose soil without peat. Within each greenhouse compartment, pots were randomly arranged in 24-pot-trays. To avoid position effects, trays were placed on tables next to each other and surrounded by "border plants" (*i.e.* plants derived by self-pollination from the study populations, sown and grown under the same conditions as the experimental plants). Trays with experimental plants were randomized twice a week until the maturation of siliques. All plants were harvested on July 1st, 2014, approximately four months after germination, when all plants that reached the flowering stage started to dry. Plants were first dried for 48h at 45°C. We then measured the dry biomass and estimated the number of siliques per plant. To estimate the number of siliques, we first separated the different branches and isolated the reproductive sections of all branches (*i.e.* the parts of the branches carrying fruits); second, we weighted the reproductive ('reproductive weight') sections of all branches of each individual together to the nearest 0.0001g using a Mettler AE 240 analytical balance. Third, we assessed the number of siliques along three randomly selected and weighted reproductive sections and estimated the number of siliques per gram ('silique density'); fourth, we estimated the total number of siliques produced per plant ('silique number') as the product of the 'silique density' and the 'reproductive weight'. This last measurement, the 'silique number', was used as a proxy for individual fecundity.

Genetic model

Traditional line cross models consider only two parental lines and generally define genetic effects by measuring the difference between parental lines and F1 to the F2 generations. Using

the F2 generations as a reference generally simplifies the mathematical expressions (Lynch & Walsh, 1998). As we aim here to analyze several line crosses at once, we reparametrized this model by taking the grand mean of the parents μ as a reference (Table S4, Figure S1). The average phenotypic means of P_i and P_j, as well as their intercrosses $F_{1,\ ij}$ and $F_{2,\ ij}$ can the expressed as:

$$P_i = \mu + A_i$$

$$P_j = \mu + A_j$$

$$F_{1,\ ij} = \mu + ½\ A_i + ½\ A_j + 2D_{ij} – AA_{ij}$$

$$F_{2,\ ij} = \mu + ½\ A_i + ½\ A_j + D_{ij} – AA_{ij}$$

This setting defines one additive effect A per parent, and as many dominance (D) and additive-by-additive (AA) epistasis parameters as independent crosses. In the absence of backcrosses, additive-by-dominance epistatic effects cannot be identified and are merged with additive effects. Dominance-by-dominance interactions, as well as higher-order epistatic terms, had to be ignored. Note that positive dominance tends to generate heterosis (F1 and F2 generations larger than the mid-parent), while positive epistasis tends to generate outbreeding depression (F1 and F2 lower than the mid-parent).

Line-cross models aim to measure individual deviations from additivity, and were thus analyzed with a fixed-effect linear model:

$$z_k = \mu + \frac{1}{2} A_{P(k)} + \frac{1}{2} A_{Q(k)} + d_k D_{P(k)Q(k)} + aa_k AA_{P(k)Q(k)} + e_k$$

for individual k of phenotype z_k, of parents from parents P(k) and Q(k), with $d_k = aa_k = 0$ if P(k) = Q(k), $aa_k = -1$ if P(k) ≠ Q(k) (k is from an intercross F_1 or F_2), and d_k = 2 (or =1) if k results from an F_1 (or an F_2) intercross. e_k is a Gaussian-distributed residual of variance V_e.

Four models of various complexity were fit to each dataset: Additive (only the additive terms A_i were considered), Dominance (A_i and D_{ij}), Epistasis (A_i and AA_{ij}), and Full model (A_i, D_{ij}, and AA_{ij}). The four models were compared by a model selection procedure based on the Akaike Information Criterion (Anderson & Burnham, 2004); AIC differences larger than 2 units were considered as a substantial discrepancy between models. Models were fit independently on Weight and Silique number, and both genetic differentiation levels (Lines and Population) were considered.

We used the best model and the associated non-additive genetic effects to simulate the consequences of non-additivity and inbreeding on the response to selection for the traits of interest, compared to an additive model. To do so, we used a two-locus and two-alleles model. We assumed infinite populations, linkage equilibrium, and genotype frequencies were always at equilibrium (accounting for selfing, i.e. the frequency of heterozygotes was p(1-p)(1-s)/(2-s) where s is the selfing rate, Hartl & Clark 1989).

RESULTS & DISCUSSION

We found that performing the analyses at the scale of the genetic lines or at the scale of the populations gave similar results, which echoes recent results arguing that the notion of population is hard to define in predominantly selfing species (Rhode & Cruzan, 2005; Dolgin *et al.*, 2007; Clo *et al.*, 2021, but see Gimond *et al.*, 2013). We decided to present the results for the genetic lines in the main text, the results at the population scale are available in supplementary materials (Table S4, Figures S2 and S3).

Consequences of hybridization on dry weight and the production of siliques

Our first question was related to the consequences of non-additive genetic effects on the phenotype of hybrid (F1 and F2) populations compared to their parents. The raw phenotypes of parental lines and within-population crosses are available as supplementary materials (Figure S3). We found that, on average, F_1 hybrids exhibited heterosis for the dry weight (Figure 1), with an increase of 9.6% (0.454g in F_1 hybrids), compared to the mean parental value of 0.414g. This heterosis level is in line with what is found in other populations of *A. thaliana* (Oakley *et al.*, 2015), and in other predominantly selfing species (Rhode & Cruzan, 2005; Dolgin *et al.*, 2007; Volis *et al.*, 2011; Gimond *et al.*, 2013; Clo *et al.*, 2021), for different fitness proxies (dry weight, fruits and seeds production). In contrast, F_2 hybrids were close to the parents (0.403g, -2%).

On the other hand, F_1 and F_2 hybrids exhibited outbreeding depression for the number of siliques (Figure 1), with respectively a decrease of 5.5% and 11.3% in F_1 and F_2 hybrids, compared to the mean parental value. This is slightly lower than other values found for different fitness proxies (seeds and fruits production, germination rate…) in other predominantly selfing species (Rhode & Cruzan, 2005; Dolgin *et al.*, 2007; Volis *et al.*, 2011; Gimond *et al.*, 2013; Oakley *et al.*, 2015; Clo *et al.*, 2021; Soto *et al.*, 2023).

These opposite patterns for dry weight and fruit number could be considered a surprising result since both traits are often considered fitness proxies, and are generally positively correlated (see Younginger *et al.*, 2017 for a review). However, such an observation is not unheard of. Studies in natural and laboratory accessions of *Arabidopsis thaliana* also found heterosis patterns for the dry mass and outbreeding depression for 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 heterosis for above- and below-ground biomass, and a slight outbreeding depression pattern for fitness (measured as the probability of bolting) in outcrossed progeny of selfing populations. Finally, Clo *et al*. (2021) found that in the predominantly selfing species *Medicago truncatula*, hybridization between inbred lines leads to heterosis for dry mass but outbreeding depression for seed production. It is known that environmental factors, such as nutrients 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). It is thus possible that the ecological conditions found in the greenhouse, which are more favorable than those in the field, might have modified trade-offs between vegetative growth and investment in reproduction; extrapolating our greenhouse results to natural populations thus relies on the assumption that environmental conditions do not affect the relative performance of genotypes (limited G×E interactions).

We also found that, on average, plants coming from low-altitude populations or hybrids generated with lines from low-altitude populations were generally heavier and produced more siliques (Figure S4), even if the differences were small. This can be explained by the difference in ecological conditions between low- and high-altitude populations, with for example mean annual temperature and precipitations being very different (see Luo *et al*., 2015 for details).

Non-additive effects in natural populations of plants

We found that non-additive effects contribute to the genetic architecture of both traits. For dry mass, we found that the best model explaining the data was the one including additive and dominant genetic effects (Table 1), and the observed pattern of heterosis was mostly due to directional positive dominant effect (d=+0.03g on average, to be compared with an average additive effect of a=0.11g, Figure 2). Oakley *et al.* (2015) found similar results in crosses between south European and Scandinavian lineages of *A. thaliana*. The directional positive dominance likely reflects the positive effects of masking deleterious mutations fixed at different loci in the different selfing lines (Charlesworth 2018). It is important to note that the contribution of epistasis to dry weight cannot totally be ruled out (Table S4).

For the number of siliques, we found that the best model explaining the data was the one including additive and additive-by-additive epistatic genetic effects (Table 1), and the observed pattern of outbreeding depression was due to directional positive additive-by-additive epistatic interactions (average aa = 83 siliques, to be compared to an average additive effect a = 262 siliques, Figure 2). The outbreeding depression can be explained by the breakdown of

positive additive-by-additive epistatic interactions found in the parental selfing lines during the hybridization events, as found in other species (Rhode & Cruzan, 2005; Johansen-Morris & Latta, 2006). The finding of directional positive epistasis is in line with what was found in other plant species (Johansen-Morris & Latta, 2006; Monnahan & Kelly, 2015; Oakley *et al.*, 2015; Clo *et al.*, 2021).

Implications for the adaptive potential of Alpine populations of A. thaliana

The distinct genetic architecture among the two fitness traits studied here implies different effects on the capacity to respond to selection. By using a two-locus and two-alleles model that mimics the genetic architecture of our traits, we explored the consequences of non-additive effects on the capacity to respond to a hypothetical selection pressure (Figure 3). When the departure to additivity is attributed to dominance, as for the dry mass, the response to selection depends on the selfing rate (Figure 3, left panel). The fact that selfing populations respond faster to selection is expected, as selfing increases the heritable variance by a factor of (1+F) due to the higher proportion of homozygotes genotypes (Wright, 1921). The fact that dominance *per se* did not affect the response to selection in selfing populations is also expected, because dominance is only expressed in heterozygote genotypes, which are rare in predominantly selfing populations/species such as *A. thaliana.* With inbreeding and directional dominance, the estimation of adaptative potential is different than in outcrossing species, because the whole genetic variance (in the different components of the genetic diversity) is a better predictor of the capacity to respond to selection than the additive variance (see for example Wright & Cockerham, 1985, Kelly 1999 or Clo & Opedal 2021 for further details). For the number of siliques, we found directional positive additive-by-additive epistasis. In the

short-term, directional positive epistasis tended to increase the capacity to respond to selection (Figure 3, right panel), as the silique production increased faster with epistasis (Figure 3, right panel). This is due to the fact that directional positive epistasis increases the amount of additive variance of a quantitative trait, and, as a result, the capacity to respond to selection (Carter *et al.*, 2005; Monnahan & Kelly, 2015). In the long term, epistasis slightly increased the seed production compared to an additive model (Figure 3, right panel), the difference being small because the epistatic coefficient is small compared to additive effects.

Limits of the statistical model to detect the nature of non-additive effects

The line cross model we proposed makes it possible to test for directional dominance and epistasis, but it has potential drawbacks. First, it can estimate directional epistasis, which is essential for understanding the capacity of populations to respond to selection, but it is not possible to dissect the different forms of epistatic variance (additive-by-additive, additive-by-dominant, and dominant-by-dominant). For inferring all these parameters from cross-line analyses, one needs more crosses than just the F_1 and F_2 individuals (see Lynch & Walsh, 1998, including reciprocal back-crosses for example, and see Oakley *et al.*, 2015 for a case study). This means that the directional positive dominance we detected for the dry weight could be a mixture of directional negative dominance and epistasis, or just complex forms of epistasis (beyond additive-by-additive interaction effects). As a consequence of this identifiability problem, genetic effect estimates for dominance and epistasis tend to be statistically correlated; in other words, the model has a substantial power to detect departure from additivity, but less power to disentangle directional dominance and epistasis.

CONCLUSIONS

Our study highlights the contribution of non-additive genetic effects to the genetic architecture of fitness components. Here, we found that both dominance and epistasis affect the genetic architecture of dry weight and silique production, leading to heterosis for the dry mass and outbreeding depression for the number of siliques in F_1 and F_2 hybrids. We however found that non-additive genetic effects remain quantitatively small compared to additive components. Simulations reflecting the genetic architecture of the studied traits, as well as the mating systems of our model species *A. thaliana*, showed that both the non-additive genetic effect and the selfing rate have a significant influence on the potential to respond to selection. Testing such theoretical predictions with artificial selection experiments is challenging (see Monnahan and Kelly, 2015; for a case study), but it appears as a necessary step to ensure whether non-additive theoretical models of adaptation are operational in practice.

Data availability: The data and code used to perform the analyses are available at https://github.com/lerouzic/Epicross.

BIBLIOGRAPHY

Abbott, R.J. & Gomes, M.F. 1989. Population genetic structure and outcrossing rate of Arabidopsis thaliana (L.) Heynh. *Heredity* **62**: 411–418.

Abu Awad, D. & Roze, D. 2018. Effects of partial selfing on the equilibrium genetic variance, mutation load, and inbreeding depression under stabilizing selection. *Evolution* **72**: 751–769.

Anderson, D. & Burnham, K. 2004. Model selection and multi-model inference. *Second. NY: Springer-Verlag* **63**: 10.

Bakerlee, C.W., Nguyen Ba, A.N., Shulgina, Y., Rojas Echenique, J.I. & Desai, M.M. 2022. Idiosyncratic epistasis leads to global fitness–correlated trends. *Science* **376**: 630–635.

Banta, J.A. & Richards, C.L. 2018. Quantitative epigenetics and evolution. *Heredity* **121**: 210–224.

Barth, S., Busimi, A.K., Utz, H.F. & Melchinger, A.E. 2003. Heterosis for biomass yield and related traits in five hybrids of Arabidopsis thaliana L. Heynh. *Heredity* **91**: 36–42.

Bergelson, J., Stahl, E., Dudek, S. & Kreitman, M. 1998. Genetic variation within and among populations of Arabidopsis thaliana. *Genetics* **148**: 1311–1323.

Carter, A.J., Hermisson, J. & Hansen, T.F. 2005. The role of epistatic gene interactions in the response to selection and the evolution of evolvability. *Theoretical population biology* **68**: 179–196.

Charlesworth, D. & Willis, J.H. 2009. The genetics of inbreeding depression. *Nature reviews genetics* **10**: 783–796.

Charlesworth, B. (2018). Mutational load, inbreeding depression and heterosis in subdivided populations. *Molecular ecology*, **27**(24), 4991-5003.

Cheverud, J.M. & Routman, E.J. 1995. Epistasis and its contribution to genetic variance components. *Genetics* **139**: 1455–1461.

Cho, L.H., Yoon, J. & An, G. 2017. The control of flowering time by environmental factors. *The Plant Journal*, **90**(4), 708-719.

Clo, J., Gay, L. & Ronfort, J. 2019. How does selfing affect the genetic variance of quantitative traits? An updated meta-analysis on empirical results in angiosperm species. *Evolution* **73**: 1578–1590.

Clo, J. & Opedal, Ø.H. 2021. Genetics of quantitative traits with dominance under stabilizing and directional selection in partially selfing species. *Evolution* **75**: 1920–1935.

Clo, J., Ronfort, J. & Abu Awad, D. 2020. Hidden genetic variance contributes to increase the short-term adaptive potential of selfing populations. *Journal of Evolutionary Biology* **33**: 1203–1215.

Clo, J., Ronfort, J. & Gay, L. 2021. Fitness consequences of hybridization in a predominantly selfing species: insights into the role of dominance and epistatic incompatibilities. *Heredity* **127**: 393–400.

Cockerham, C.C. & Weir, B.S. 1984. Covariances of relatives stemming from a population undergoing mixed self and random mating. *Biometrics* 157–164.

Crow, J.F. 2010. On epistasis: why it is unimportant in polygenic directional selection. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**: 1241–1244.

Dolgin, E.S., Charlesworth, B., Baird, S.E. & Cutter, A.D. 2007. Inbreeding and outbreeding depression in Caenorhabditis nematodes. *Evolution: International Journal of Organic Evolution* **61**: 1339–1352.

Falconer, D.S. & Mackay, T.F.C. 1996. Introduction to quantitative genetics. Essex. *UK: Longman Group*.

Gimond, C., Jovelin, R., Han, S., Ferrari, C., Cutter, A.D. & Braendle, C. 2013. Outbreeding depression with low genetic variation in selfing Caenorhabditis nematodes. *Evolution* **67**: 3087–3101.

Hansen, T.F. 2015. Measuring gene interactions. *Epistasis* 115–143. Springer.

Hansen, T.F. 2013. Why epistasis is important for selection and adaptation. *Evolution* **67**: 3501–3511.

Hartl, D. L., & Clark, A. G. 1989. Principles of population genetics. Sinauer Assoc. *Inc, Sunderland, Massachusetts*.

Hoffmann, M.H. 2002. Biogeography of Arabidopsis thaliana (l.) heynh.(Brassicaceae). *Journal of Biogeography* **29**: 125–134.

Johansen-Morris, A.D. & Latta, R.G. 2006. Fitness consequences of hybridization between ecotypes of Avena barbata: hybrid breakdown, hybrid vigor, and transgressive segregation. *Evolution* **60**: 1585–1595.

Kelly, J.K. 1999. Response to selection in partially self-fertilizing populations. I. Selection on a single trait. *Evolution* **53**: 336–349.

Kelly, J.K. & Arathi, H.S. 2003. Inbreeding and the genetic variance in floral traits of Mimulus guttatus. *Heredity* **90**: 77–83.

Kouyos, R.D., Silander, O.K. & Bonhoeffer, S. 2007. Epistasis between deleterious mutations and the evolution of recombination. *Trends in ecology & evolution* **22**: 308–315.

Lande, R. & Porcher, E. 2015. Maintenance of quantitative genetic variance under partial self-fertilization, with implications for evolution of selfing. *Genetics* **200**: 891–906.

Le Rouzic, A. 2014. Estimating directional epistasis. *Frontiers in Genetics* **5**: 198.

Li, Y., van Kleunen, M. & Stift, M. 2019. Sibling competition does not magnify inbreeding depression in North American Arabidopsis lyrata. *Heredity* **123**: 723–732.

Luo, Y., Widmer, A. & Karrenberg, S. 2015. The roles of genetic drift and natural selection in quantitative trait divergence along an altitudinal gradient in Arabidopsis thaliana. *Heredity* **114**: 220–228.

Lynch, M. & Walsh, B. 1998. *Genetics and analysis of quantitative traits*. Sinauer Sunderland, MA.

Maisnier-Patin, S., Roth, J.R., Fredriksson, Å., Nyström, T., Berg, O.G. & Andersson, D.I. 2005. Genomic buffering mitigates the effects of deleterious mutations in bacteria. *Nature Genetics* **37**: 1376–1379.

Monnahan, P.J. & Kelly, J.K. 2015. Epistasis is a major determinant of the additive genetic variance in Mimulus guttatus. *PLoS Genet* **11**: e1005201.

Nasrallah, M.E., Yogeeswaran, K., Snyder, S. & Nasrallah, J.B. 2000. Arabidopsis species hybrids in the study of species differences and evolution of amphiploidy in plants. *Plant Physiology* **124**: 1605–1614.

Oakley, C.G., Agren, J. & Schemske, D.W. 2015. Heterosis and outbreeding depression in crosses between natural populations of Arabidopsis thaliana. *Heredity* **115**: 73–82.

Pavlicev, M., Le Rouzic, A., Cheverud, J.M., Wagner, G.P. & Hansen, T.F. 2010. Directionality of epistasis in a murine intercross population. *Genetics* **185**: 1489–1505.

Pélabon, C., Albertsen, E., Le Rouzic, A., Firmat, C., Bolstad, G. H., Armbruster, W. S., & Hansen, T. F. (2021). Quantitative assessment of observed versus predicted responses to selection. *Evolution*, *75*(9), 2217-2236.

Phillips, P.C., Otto, S.P., Whitlock, M.C., Wolf, J.D., Brodie, E.D.I. & Wade, M.J. 2000. Beyond the average: the evolutionary importance of gene interactions and variability of epistatic effects. *Epistasis and the evolutionary process* 20–38.

Picó, F.X., Méndez-Vigo, B., Martinez-Zapater, J.M. & Alonso-Blanco, C. 2008. Natural genetic variation of Arabidopsis thaliana is geographically structured in the Iberian peninsula. *Genetics* **180**: 1009–1021.

Rhode, J.M. & Cruzan, M.B. 2005. Contributions of heterosis and epistasis to hybrid fitness. *The American Naturalist* **166**: E124–E139.

Rieseberg, L.H., Archer, M.A. & Wayne, R.K. 1999. Transgressive segregation, adaptation and speciation. *Heredity* **83**: 363–372.

Rieseberg, L.H., Widmer, A., Arntz, A.M. & Burke, B. 2003. The genetic architecture necessary for transgressive segregation is common in both natural and domesticated populations. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **358**: 1141–1147.

Shaw, R.G., Byers, D.L. & Shaw, F.H. 1998. Genetic components of variation in Nemophila menziesii undergoing inbreeding: morphology and flowering time. *Genetics* **150**: 1649–1661.

Soto, T. Y., Rojas-Gutierrez, J. D., & Oakley, C. G. 2023. Can heterosis and inbreeding depression explain the maintenance of outcrossing in a cleistogamous perennial?. *American Journal of Botany*, in press.

Sztepanacz, J. L., Clo, J., & Opedal, Ø. H. (2023). Evolvability, Sexual Selection, and Mating Strategies. in *Evolvability, a unifying concept in evolutionary biology?*

Vasseur, F., Fouqueau, L., De Vienne, D., Nidelet, T., Violle, C. & Weigel, D. 2019. Nonlinear phenotypic variation uncovers the emergence of heterosis in Arabidopsis thaliana. *PLoS biology* **17**: e3000214.

Volis, S., Shulgina, I., Zaretsky, M. & Koren, O. 2011. Epistasis in natural populations of a predominantly selfing plant. *Heredity* **106**: 300–309.

Walsh, B. & Lynch, M. 2018. *Evolution and selection of quantitative traits*. Oxford University Press.

Wright, A.J. & Cockerham, C.C. 1985. Selection with partial selfing. I. Mass selection. *Genetics* **109**: 585–597.

Younginger, B.S., Sirová, D., Cruzan, M.B. & Ballhorn, D.J. 2017. Is biomass a reliable estimate of plant fitness? *Applications in Plant Sciences* **5**: 1600094.

Table 1. Summary of the statistical models fitted to data, when analyses are performed at the scale of genetic lines, for the dry weight and the number of siliques. In the table, "a" stands for additive, "d" for dominance, and "aa" for additive-by-additive epistasis. ΔAIC is the difference in AIC values between the observed and best models, differences of 2 AIC units or more are generally considered as solid statistical support for the best model.

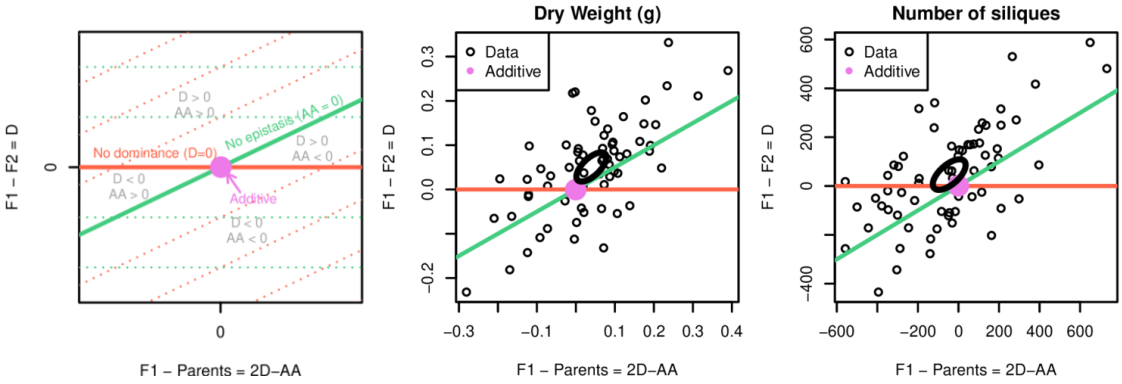
Trait	Model	Log(likelihood)	d.f.	ΔAIC
Dry weight	a	535.20	28	60.84
	a.d	630.62	93	0.00
	a.aa	625.43	91	6.38
	a.d.aa	683.88	151	9.48
Number of silliques	a	-10729.13	28	98.10
	a.d	-10624.23	93	18.31
	a.aa	-10617.08	91	0.00
	a.d.aa	-10574.11	151	34.06

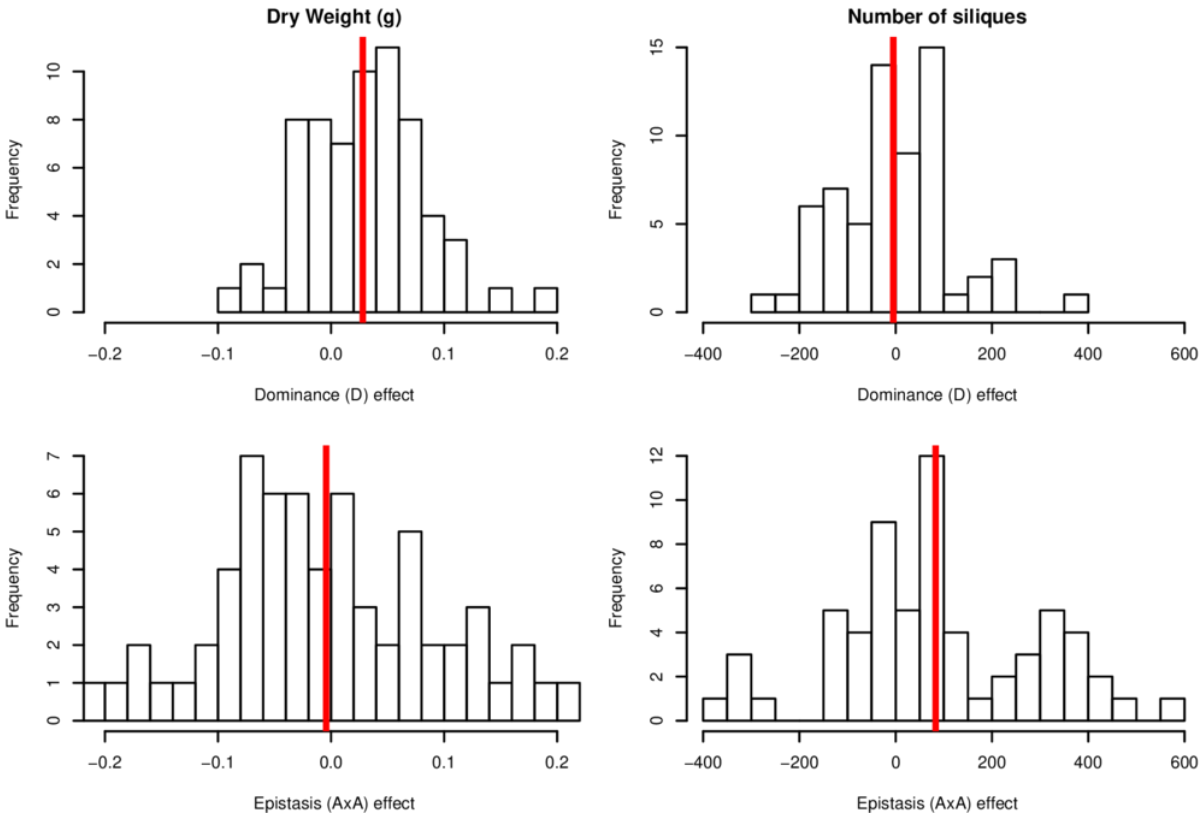
Figure captions:

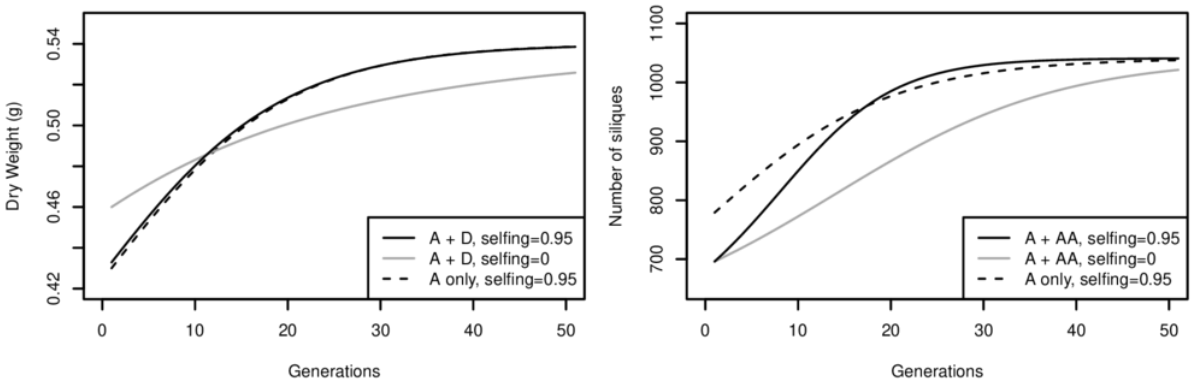
Figure 1. Graphical representation of the parental, F_1 and F_2 values, when analyses are performed at the scale of the genetic lines. **Left panel.** Empty representation indicating the directionality of dominance and epistasis as a function of the position of the datapoints. **Middle panel.** Distribution of data for the dry weight. **Right panel.** Distribution of data for the number of siliques. The ellipse represents the 95% confidence ellipse of the average across all crosses.

Figure 2. Distribution of the dominance and epistatic genetic effects when analyses are performed at the scale of the genetic lines, for the dry weight and the number of siliques. The red lines are indicating mean values.

Figure 3. Simplified simulations of hypothetical responses to directional selection on dry weight (left) and number of siliques (right). The additive case (hyphenates lines) is contrasted to two-allele two-locus genetic architectures in which the relevant non-additive effects are accounted for (dominance for dry mass, epistasis for number of siliques; effects were averaged out over all pairs of populations). We assumed infinite populations, linkage equilibrium, and genotype frequencies were always at equilibrium (accounting for selfing, i.e. the frequency of heterozygotes was p(1-p)(1-s)/(2-s) where s is the selfing rate, Hartl & Clark 1989). The selfing rate in *A. thaliana* is about 0.95; simulations with random mating populations (gray) are provided for comparison. Fitness was proportional to the phenotype; the lowest fitness genotype was 80% that of the best.


F1 – F2 = D
F1 – Parents = 2D–AA
No dominance (D=0)
No epistasis (AA = 0)
Additive
D > 0
AA > 0
D > 0
AA < 0
D < 0
AA > 0
D < 0
AA < 0
Dry Weight (g)
Number of siliques
Data
Additive


Dry Weight (g)
Number of siliques
Frequency
Dominance (D) effect
Epistasis (AxA) effect


Dry Weight (g)
0.42
0.46
0.50
0.54
0
10
20
30
40
50
Generations
A + D, selfing=0.95
A + D, selfing=0
A only, selfing=0.95
Number of siliques
700
800
900
1000
1100
0
10
20
30
40
50
Generations
A + AA, selfing=0.95
A + AA, selfing=0
A only, selfing=0.95

548