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Does seed size respond to selection on blossom size?

An artificial selection experiment on
Dalechampia scandens

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MSc in Biology

Submission date: May 2017

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Abstract

1. Within populations variation in seed size may result from phenotypic correlation between seed size and flower size, a hypothesis originally suggested by Primack (1987). Such phenotypic correlation can be generated either by environmental or genetic covariation among traits. If phenotypic correlation between seed size and flower size results from genetic covariation, this may strongly affect the evolution of both traits.

2. I investigated the correlation between seed size and flower size using an artificial selection experiment performed on two populations of *Dalechampia scandens* selecting for larger and smaller blossoms for 4 generations. In the last generation, blossoms and seeds were measured in order to estimate the direct and correlated response to selection and infer the genetic regression of seed size on blossom size. In order to estimate the coefficient of variance, seeds and blossoms were measured in the control line

3. Seed size increased by 0.20% and 0.14% per percent increase in blossom size due to selection, in the two populations. The coefficient of variance was considerably lower for seed size compared to blossom size.

4. Because seed size responded little to selection on blossom size, and because seed size varies considerably less than size of floral structures, I consider seed size to be genetically canalized.

Sammendrag

1. Variasjon i frøstørrelse innad i populasjoner kan resultere fra fenotypisk korrelasjon mellom frøstørrelse og blomstørrelse, en hypotese opprinnelig foreslått av Primack (1987). Fenotypisk korrelasjon kan genereres enten ved miljø- eller genetisk kovarians mellom egenskaper. Dersom fenotypisk korrelasjon mellom frøstørrelse og blomstørrelse skyldes genetisk kovarians, kan dette påvirke evolusjonen av begge egenskapene.
2. Jeg utforsket korrelasjonen mellom frøstørrelse og blomstørrelse ved å benytte meg av et seleksjonsekperiment utført på to populasjoner av *Dalechampia scandens*, hvor det ble selektert for større og mindre blomster over 4 generasjoner. I den siste generasjonen ble blomster og frø målt for å estimere den direkte og korrelerte responsen til seleksjon, og for å estimere den genetiske regresjonen av frøstørrelse på blomstørrelse. For å estimere varianskoeffisienten ble frø og blomster målt i kontroll linjen.
3. Frøstørrelse økte med 0.20% og 0.14% per prosent økning i frøstørrelse grunnet seleksjon, i de to populasjonene. Varianskoeffisienten var betraktelig lavere for frøstørrelse sammenlignet med blomstørrelse.
4. Fordi frøstørrelse responderte lite til seleksjon på blomstørrelse, og fordi frøstørrelse varierte betraktelig mindre enn størrelsen på strukturer i blomsten, betrakter jeg frøstørrelse som kanalisert.

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Introduction

Seed size is an important life-history trait affecting germination, seedling establishment, and seedling survival (Westoby *et al.* 1996; Vaughton & Ramsey 1998; Leishman & Wright 2000). Among species, seed size ranges over more than 10 orders of magnitude (Harper *et al.* 1970), reflecting the great variety of plant life-history strategies (Stearns 1992). Within populations, the positive effect of offspring size on survival combined with the trade-off between offspring size and offspring number for mothers with limited resources is expected to generate an optimum size that maximizes maternal fitness (Smith & Fretwell 1974). This optimum is expected to generate stabilizing selection on seed size which should be canalized (McGinley *et al.* 1987), so that individuals within a population produce constant phenotype independently of genetic and/or environmental variation (Flatt 2005).

However, seed size varies considerably within species and also within populations (Michaels *et al.* 1988; Obeso 1993; Vaughton & Ramsey 1998). One possible explanation such the within-population variation is that seed size covaries with other phenotypic traits (McGinley *et al.* 1987). Several hypotheses have been suggested to explain covariation. First, the covariation may result from phenotypic integration, the covariation of phenotypic traits under genetic or environmental perturbations (Armbruster *et al.* 2014). Phenotypic integration may result from the necessity of traits that interact functionally to covary in order to preserve the proper functioning of the system. This adaptive explanation, initially suggested by Olson & Miller (1958) is referred to as functional integration. Because flowers are functionally integrated units (Gómez *et al.* 2009), where seeds develop surrounded by maternal tissues forming the gynoeceium (Linkies *et al.* 2010), seed size may be expected to correlate with flower size. This was suggested by Primack (1987) to explain among species correlation in seed size and flower size. This phenotypic integration should result from genetic correlation with flower size, resulting from either linkage disequilibrium or pleiotropy. Alternatively, phenotypic integration may result from developmental constraints (e.g. traits sharing part of their developmental pathway; Klingenberg (2008)). In this case, if the correlated traits are under different selection pressures, the correlation may constrain adaptive evolution in one or both traits (Hansen & Houle 2008; Agrawal & Stinchcombe 2009; Bolstad *et al.* 2014). If seed size and blossom size are under different selection pressures, such integrations may constrain adaptive evolution of seed size towards the optimum, and inhibit canalization.

Another source of phenotypic covariation could be that environmental variation among or within individuals affects the traits simultaneously. Among plant differences in resource availability may affect both seed size and flower size (Sakai & Sakai 1995). Within a plant, the position of the flower may also affect both flower size and seed size, either due to micro-environmental variation (Roach & Wulff 1987), or due to variation in stem diameter that is expected to affect the size of the organ it carries, because stem diameter affects the amount of resources that can be transferred (Niklas 1994). Therefore, the reserves available for both flower and developing seeds may covary, depending on the diameter of the stem.

Overall, phenotypic correlations among individuals include genetic and environmental sources of covariation (Searle 1961), while phenotypic correlation within an individual, estimated by repeated measurements of modular organs such as flowers, should only reflect micro-environmental and positional sources of covariation (Klingenberg 2014). It has been suggested that genetic correlations among traits can be approximated by phenotypic correlations (Cheverud 1988; Waitt & Levin 1998). However, this may not be the case if the environmental covariation is in the opposite direction of the genetic covariation (Hadfield *et al.* 2007; Pélabon *et al.* 2013), or if variation in the environment generates independent variation in both traits and masks the genetic signal (Hadfield *et al.* 2007; Pélabon *et al.* 2013). As a consequence, genetic correlations inferred from phenotypic correlations may not be accurate (Reznick 1985).

An efficient way of estimating genetic correlations is to conduct artificial selection experiments (Reznick 1985; Conner 2003; Roff 2007). As shown by Lande (1979), if two traits X and Y are genetically correlated, selection only on trait X should generate a correlated response in trait Y. The bivariate mean should evolve along the line defined by the slope of the genetic regression of Y on X: $\beta = Cov(X, Y) / Var(X)$, where $Cov(X, Y)$ is the genetic covariance between X and Y and $Var(X)$ is the genetic variance in X (Fig. 1). By considering trait values on a logarithmic scale, the regression line can be interpreted as an allometric line, describing the proportional change in trait Y due to a proportional change in X. If the traits are closely connected, we expect the slope of the regression line to be close to 1 (isometry). However, for traits that are largely decoupled, we expect the regression to have slope close to 0.

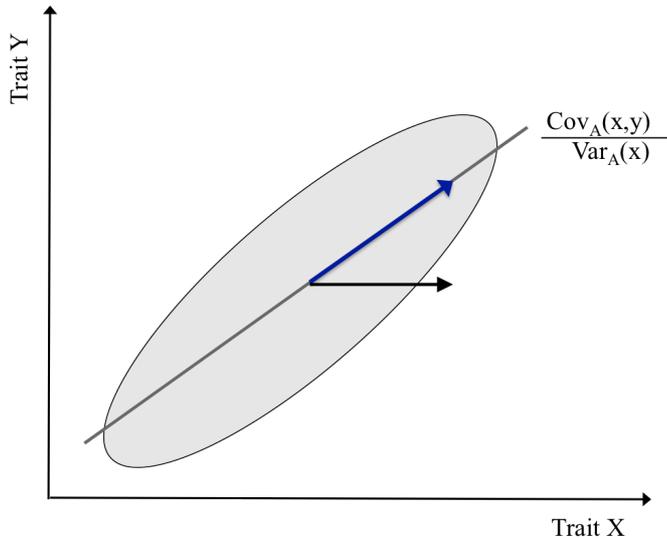


Figure 1. Correlated response in trait Y (blue arrow) to selection on trait X (black arrow) follows the genetic regression of trait Y on X (grey line). The slope of the regression is the ratio of covariance between the traits ($Cov(X,Y)$) to variance in trait X ($Var(X)$).

Surprisingly, very few studies have tested Primack's hypothesis for correlations between blossom size and seed size (Primack 1987). Lehtilä & Holmn Bränn (2007) found a negative genetic correlation between petal size and seed size. However, they did not control for variation in seed number, which could be a confounding factor (Smith & Fretwell 1974).

In this study, we take advantage of an artificial selection experiment performed on a floral character in *Dalechampia scandens* for 4 generations, to examine the genetic correlation between the blossom size and seed size. The selection was performed on one trait in the blossom, where the whole blossom responded to the selection. A correlated response in seed size due to selection on blossom size would provide evidence for a genetic correlation between the traits. In contrast, if seed size does not respond to selection on blossom size, the traits are decoupled. If seed size is a genetically canalized trait, it should remain constant despite of genetic perturbation. Further, by comparing the phenotypic variance in seed size and blossom size, I investigate whether seed size could be a genetically canalized trait.

Dalechampia scandens is a particularly suitable species for this study. First, blossom size and seed size varies within and among populations. Additionally, individuals produce many blossoms simultaneously throughout their life, and seeds are large and easy to measure. Each blossom produces a constant number of seeds, so that the trade-off between size and number can easily be controlled for.

Methods

STUDY SPECIES AND TRAIT MEASUREMENTS

Dalechampia scandens L. (Euphorbiaceae) is a perennial vine distributed from Mexico to Argentina (Armbruster 1985). It is monoecious, with male and female flowers aggregated into bisexual inflorescence. Each inflorescence contains three female flowers located below a cluster of 10 male flowers and a resin-producing gland, subtended by two involucre bracts (Webster & Webster 1972; Bolstad *et al.* 2014). Each female flower contains three ovules, so that a blossom can produce a maximum of nine seeds (Armbruster 1982). Each blossom has a receptive period of 7-10 days, during which it opens for 3-4 hours per day. The first 2-3 days only female flowers are receptive, while the male flowers remain closed. Subsequently follows a period of 5-6 days when male flowers open and the stigma may receive pollen from the anthers of the same blossom (autogamous selfing) (Armbruster 1982, 1985).

The resin-producing gland is ecologically important, because it produces the reward for pollinating bees that use the resin in nest construction (Armbruster 1984). The size of the gland affects the amount of resin produced (Pélabon *et al.* 2012), and consequently the maximum size of the pollinators that can afford to visit the blossoms (Armbruster 1984; Armbruster & Herzig 1984), as a result of the energy-cost/resin-benefit balance (Heinrich & Raven 1972). The bracts function as signal to the pollinating bee (Pérez-Barrales *et al.* 2013), but also protects the blossom at night by closing around the flowers and the resin-producing gland during the fertile period, and by closing permanently around the developing seeds. At maturation, the seeds are dispersed by explosive dehiscence, which is a crucial stage requiring a very precise mechanism where the proportional sizes of the structures involved may be particularly important (Armbruster 1982). The different structures composing the blossom act as an integrated unit, tied together by strong genetic and phenotypic correlations (Hansen *et al.* 2003; Bolstad *et al.* 2014). The selection experiment from which the data of this study were recorded consisted of selecting the resin-producing gland for an increase or a decrease in size. Because of the high degree of integration among the floral traits in the *Dalechampia* blossom, selection in gland size generated correlated response in the whole blossom size as illustrated by the correlated response of the bract size (Albertsen & Pélabon unpublished data). Therefore, we hypothesize that selection on gland area will generate response of all traits correlated.

In this study, we used two populations belonging to two different undescribed species of the *D. scandens* species complex (Bolstad *et al.* 2014; Falahati-Anbaran *et al.* 2017). These two populations differ by the size of their blossoms and also the size of their seeds (Table 1).

Table 1. GPS-coordinates and mean \pm SE size of seeds (mm), gland ($\sqrt{\text{gland area}}$ in mm) and bract ($\sqrt{\text{bract area}}$ in mm) in the Tulum and Tovar populations. The estimates of seed size come from this study, and the estimates of gland size and bract size are from Bolstad *et al.* (2014).

Population	Coordinates	Avg. seed size (mm) \pm SE	Avg. $\sqrt{\text{gland area}}$ (mm) \pm SE	Avg. $\sqrt{\text{bract area}}$ (mm) \pm SE
Tulum	20.13 N, 87.26 W	4.11 \pm 0.18	4.37 \pm 0.04	38.19 \pm 0.34
Tovar	8.21 N, 71.46 W	3.26 \pm 0.12	4.15 \pm 0.06	37.94 \pm 0.60

Selection experiment

The selection experiment was conducted in the greenhouse at the Department of Biology at NTNU. Seeds were originally collected near Tulum in the state of Quintana Roo, Mexico and near Tovar in the state of Mérida, Venezuela in 1998 (Table 1). The two populations have been subjected to four episodes of selection for increased and decreased gland size. Three lines were maintained within each population: up selected (to increase gland area), down selected (to decrease gland area), and a control line. The methods of the selection experiment are presented in detail in Albertsen *et al.* (in prep), and here I provide only a summary of the protocol.

The experiment was started by C. Pélabon in 2006. The parental populations comprised ca. 200 individuals produced by one or two episodes of outcrossing of individuals grown from seeds collected in the natural populations. Therefore, these were the second or third greenhouse generation. For each individual in the parental population, 3 to 4 blossoms were measured (all measurements done by C. Pélabon). The 16 individuals with the largest and smallest average gland size were selected. Each selected individual was crossed four times to obtain 64 families. The pedigree was recorded and individuals that were the least related were crossed to maintain inbreeding at the lowest possible level in the subsequent generations. At each generation, additional crosses were performed to ensure that each plant contributed equally to the next generation. Two seeds per cross were then sown and one individual randomly selected to form the F1 generation. From this generation onwards, 16 individuals with the largest (up selected line) or smallest (down selected line) average gland size were

selected at each generation and crossed four times, to build the next generation of 64 individuals. Other blossom traits have been measured, and their correlated response to selection will be presented elsewhere.

Mature seeds from the selected individuals at each generation have been stored in paper envelopes in a dry environment at NTNU. Seed diameter (mm) and gland area (length \times average of height on each side, mm²) were measured with digital calipers (0.01mm precision). Seed diameter is a better measure of seed size than seed mass, because this latter may vary temporally due to water loss. Seed mass was measured for a subset of seeds to test the relationship between seed mass and diameter ($r^2 = 0.85$, $n = 424$). Repeated measurements on seed diameter were conducted over the whole experiment to estimate measurement error. The repeatability of the measurements was very high ($r^2 = 0.996$, $n = 2153$), indicating very low measurement error for this trait. I measured only seed sets with more than six seeds to minimize variation in seed size due to variation in seed number. Furthermore I only measured complete seed sets using the extra seed set produced at each generation in each line. To be able to compare seed size and gland size on the same scale (in mm), gland area was square root transformed before analyses. I refer to this measure in mm as gland size.

Response to selection

The direct response to selection in gland size and the correlated response in seed size were estimated by measuring both traits in the last generation (F4). Gland size was measured by E. Albertsen for the Tovar population, and C. Pélabon for the Tulum population. Observer effects are not a concern here because both the up and down selection lines were measured by the same observer in each population. To measure seed size, I conducted random crosses in each population in the up and down selected lines to produce seeds. I performed one cross for each of 60 and 58 individuals for Tulum and Tovar, respectively, in November/December 2016 and January/February 2017. Sample sizes are presented in Table A1, Appendix.

Within-individual environmental regression

A single blossom is subject to two different levels of environmental variation: the variation in the environment surrounding the plant, affecting the whole individual, and the micro-environmental variation due to the position of the blossom on the plant (Herrera 2009). The latter will affect resource allocation within the plant (Roach & Wulff 1987) and will generate

correlation among traits that are only due to environmental variation. Indeed, correlation among traits at the among-individual level confounds environmental and genetic variation. We estimated phenotypic correlations between gland size and seed size at both within and among individual levels.

To estimate the environmental correlation between gland size and seed size within individuals, crosses were performed in the control lines in the F3 and F4 generations for Tulum and Tovar, respectively. On the first day of the bisexual phase, blossoms were self-pollinated by transferring pollen from a freshly dehisced male flower to the three stigmas. The male cymule was removed with a scalpel before gland measurements. Finally, I placed a thin paper bag around the blossom to collect seeds at maturation. Up to four crosses were performed per individual, with 29 individuals in Tulum ($n = 102$ crosses) and 28 in Tovar ($n = 80$ crosses). Crosses were conducted in April and November/December 2016 for Tulum and Tovar, respectively. Self-fertilization may affect the size of the seeds (Armbruster & Rogers 2004, but see ; Opedal *et al.* 2015), but we do not expect it to affect the relationship between gland size and seed size. After harvesting the seeds, I measured the diameter of the seeds produced (Tulum: 900 seeds, Tovar: 708 seeds). These measurements were also used to estimate the square coefficient of variance.

Phenotypic regression across individuals

From the selection experiment, measurements of gland area for three blossoms per individual were available as well as seeds from the selected individuals in all lines and generations. During the autumn 2015 and spring 2016, I measured one seed set for each of the 16 selected plants at each generation [number of seed sets (seeds); Tulum: $n = 128$ (1101), Tovar: $n = 129$ (1052)] (see Table A2, Appendix, for details on sample size). I only included complete seed sets to avoid the possible effect of seed selection when sowing the seeds. Although seed sets from the same father may have been measured, this should not affect the results because paternal effects are very weak in *Dalechampia scandens* (Pélabon *et al.* 2015). Seed sets from the different lines and generations were measured at random to avoid the confounding effect of increasing measuring skills or time of measurements.

STATISTICAL ANALYSES

Response to selection

Because the genetic variation in seed size in the parental population has not been estimated, it was not possible for me to calculate the genetic correlation between gland size and seed size. Instead, I tested the relationship between blossom size (as estimated by the gland size) and seed size by estimating the slope of the genetic regression of seed size on gland size. This slope can be estimated by the ratio between the correlated response to selection and the direct response to selection:

$$\beta_G = \frac{\text{Correlated response to selection}}{\text{Direct response to selection}} = \frac{\Delta \text{Seed size}}{\Delta \text{Gland size}} \quad (\text{Eq. 1})$$

where the direct and correlated responses are calculated as the difference in average gland size and seed size between the up and down selected lines in the last generations. When estimated on log-transformed data, the genetic regression slope can be interpreted as an allometric slope, representing the proportional evolutionary change in seed size for a proportional evolutionary change in gland size (Lande 1979). I estimated the direct and correlated response to selection by fitting mixed-effect models (Bates *et al.* 2015) with either seed size or gland size as response variables, line as fixed effect and plant identity as random factor. Standard errors for the slopes were estimated using the formula for variance for ratios by Lynch & Walsh (1998). All statistical analysis were performed in R Software version 3.3.3 (R Core Team 2017).

Components of phenotypic variance

I examined the level at which variation in seed size was generated by fitting a mixed-effect model with seed size as response variable and blossom nested within plant identity as random factor. To compare between-blossom and between-individual variation in seed size and gland size, I used two mixed-effect models with either average seed size within blossom or gland size as response variables, and plant identity as random factor. The variance components were mean-square standardized and multiplied by 100 to obtain the square coefficient of variation (CV^2) as a measure of proportional variation.

Within-individual environmental regression

To compare the slope of the genetic regression estimated from the direct and correlated response to selection with the slope generated by environmental covariation, I estimated the regression between average seed size and gland size at the blossom level after mean-centering both variables on individual means, with all data log-transformed. I fitted a mixed-effect model with mean-centered log seed size as response variable, and mean-centered log gland area as fixed effect. Plant identity was treated as a random factor.

Phenotypic regression across individuals

Finally I compared the genetic regression slope with the regression slope of seed size on gland size across individuals in all generation and all lines. I fitted a mixed-effect model with log seed size (average of one seed set per individual) as response variable, log gland size (average of three glands per individual) as fixed effect, and a dummy variable combining lines and generations as random factor.

Results

Response to selection

Gland size differed by $16.49 \pm 2.05\%$ (mean \pm SE from here and onwards) and $23.70 \pm 1.15\%$ between the up and down selected line in the last generation (F4) in Tulum and Tovar, respectively (Fig. 2). In comparison, seed size differed by $3.53 \pm 0.80\%$ between the up selected line (mean seed diameter: 4.31 ± 0.02 mm) and the down selected line (4.16 ± 0.02 mm) in the Tulum population. In Tovar, seed size differed by $3.38 \pm 1.03\%$ between the up selected line (3.36 ± 0.02 mm) and the down selected line (3.24 ± 0.03 mm) (Fig. 2). The slope of the genetic regression of log seed size on log gland size was $0.201 \pm 0.055\%$ and $0.143 \pm 0.044\%$ in Tulum and Tovar, respectively (Fig. 4).

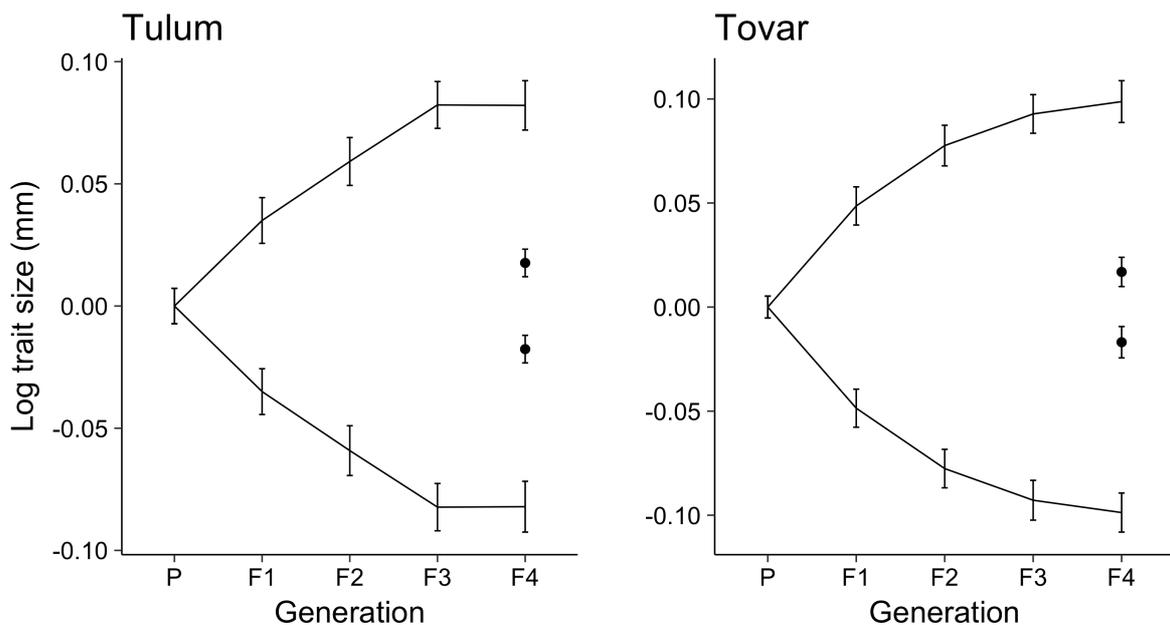


Figure 2. Effect of selection across generations on log gland size (log mm) and log seed size (log mm) (points at the F4), as mean \pm 1 SE. Data are grand mean centered at each generation. Sample sizes are presented in Table A1, Appendix for generation F4 and Table A2, Appendix for the other generations.

Components of phenotypic variance

The squared coefficient of variation (CV^2) was considerably higher for gland size than for seed size (Table 2). For both traits, a higher proportion of the total variance was generated within individuals, rather than among. Within-individual CV^2 of seed size was similar in the two populations, but CV^2 was smaller among individuals in Tulum than in Tovar. The CV^2 of gland size indicates that, in the Tovar population, variation among-individuals was more pronounced than in the Tulum population.

Table 2. Squared coefficient of variance (CV^2) and percentage of total variance generated among individuals, among blossoms and within blossoms in seed size, and among individuals and blossoms in gland size.

Population	Level of variance	Seed size		Gland size	
		CV^2	% of tot. variance	CV^2	% of tot. variance
Tulum	Among individuals	0.018	20.4	0.22	23.0
	Among blossom	0.040	49.6	0.74	77.0
	Within blossom	0.026	30.0	-	-
	Sum	0.084		0.96	
Tovar	Among individuals	0.045	43.0	0.32	36.5
	Among blossoms	0.038	36.9	0.56	63.5
	Within blossom	0.021	20.1	-	-
	Sum	0.104		0.88	

Within-individual environmental regression

Within individuals, seed size increased by $0.053 \pm 0.02 \%$, and $0.01 \pm 0.03\%$ per percentage increase in gland size in Tulum and Tovar, respectively (Fig. 3). These regression slopes were shallower than the genetic regressions, indicating that within plant environmental variation has a very weak effect on correlation between gland size and seed size.

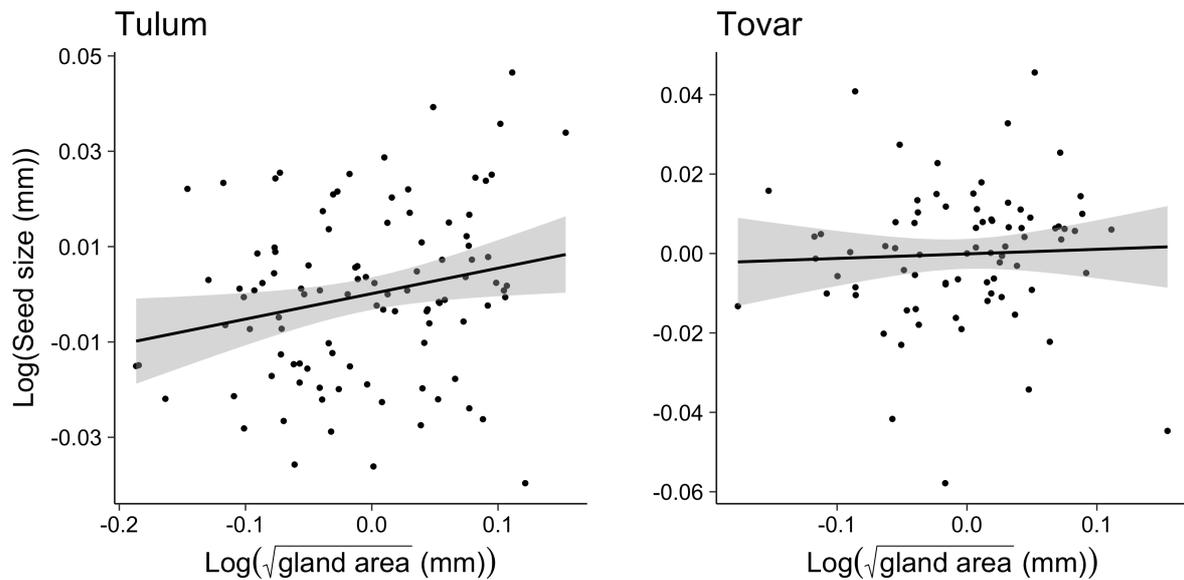


Figure 3. Effect of gland size (log mm) on average seed size (log mm) across blossoms, centered on individual mean with standard errors. $n = 102$ blossoms for Tulum and $n = 81$ blossoms for Tovar.

Phenotypic regression across individuals

Across individuals in all generations and lines, seed size increased by $0.132 \pm 0.04\%$ SE and $0.142 \pm 0.04\%$ per percent increase in gland size for Tulum and Tovar, respectively (Fig. 4). In Tulum, the phenotypic regression across individuals was shallower compared to the genetic slope, indicating that environmental variation masks the genetic slope. In Tovar, the slopes of the phenotypic and genetic regressions were similar. The overall positive relationship between gland size and seed size was also evident within generations, where most regressions were positive (Fig. 4).

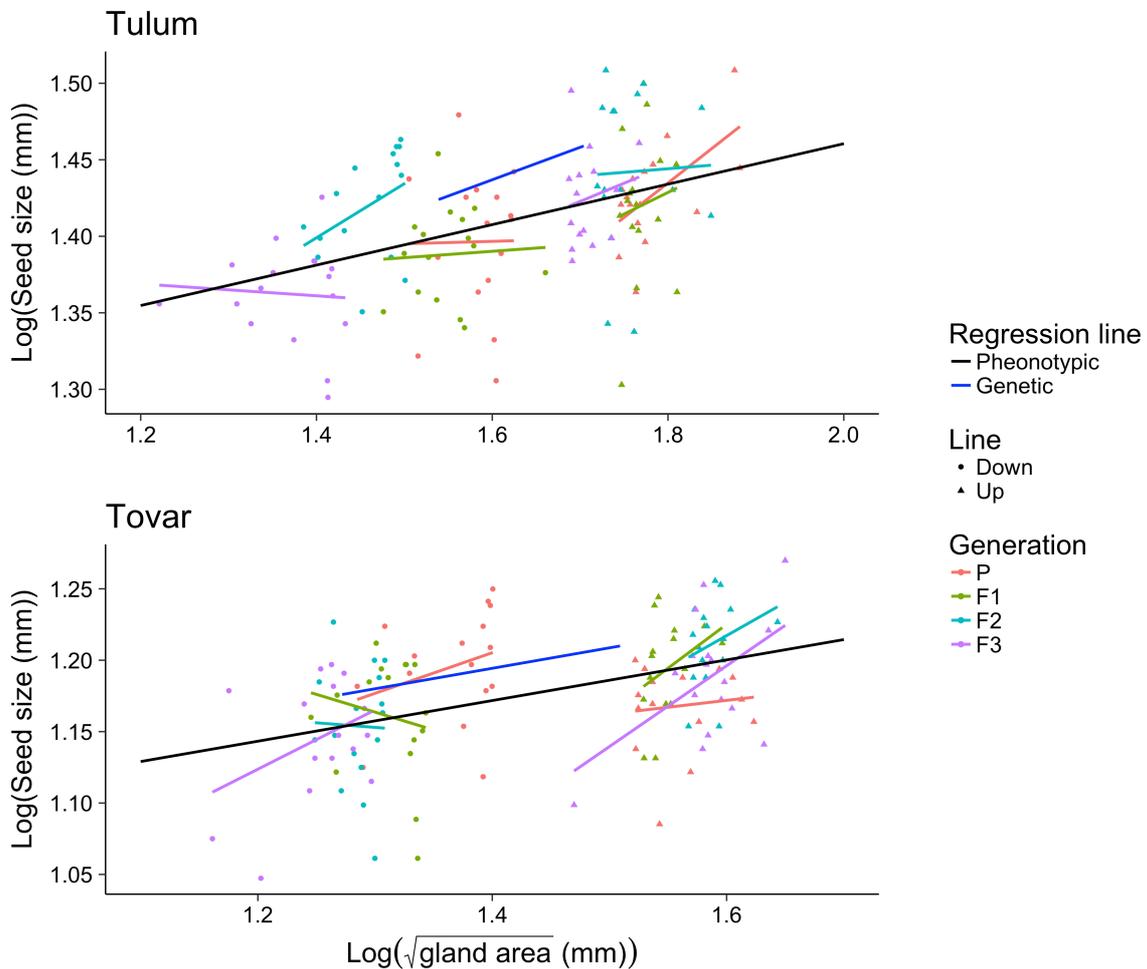


Figure 4. Effect of log individual average gland size (log mm) on log average seed size (log mm) in all generations (different colors) of both selection lines (up selected line represented by triangles and down selected by circles), the phenotypic regression across all individuals (black line), and the genetic regression (blue line) derived from the correlated and direct response to selection in F4. Sample sizes are presented in Table A2, Appendix.

Discussion

In this study, I tested whether seed size is phenotypically and/or genetically correlated with blossom size in *Dalechampia scandens*. After 4 episodes of selection for larger and smaller gland, this character differed by 16% and 23% between up and down selected lines in Tulum and Tovar, respectively. Although this selection generated some correlated response on the whole blossom size (Albertsen, unpublished data), the correlated response of seed size caused a difference of about 3% between the selection lines in both populations. Thus, an increase in gland size of one percent due to selection corresponded to an increase of 0.2% and 0.142% in seed size in Tulum and Tovar, respectively. In Tulum, there was a weak effect of gland size on seed size within individuals, although the effect size was small ($0.05 \pm .02$ %), while in Tovar, I found no within-individual effect. Individuals that produced larger blossoms also produced larger seeds, and the slopes of the phenotypic regressions across individuals were weaker or the same as the slopes of the genetic regressions, for Tulum and Tovar respectively. Also, seed size varied considerably less than blossom size.

Due to the trade off between seed size and number, selection on seed size may alter the number of seeds produced. In the only previous study I know of looking at the response in seed size to selection on blossom size (pollinator attracting trait), they found a negative genetic correlation between petal and seed size (Lehtilä & Holm Bränn 2007). However, large blossoms produced more seeds, and the total seed mass a plant produced remained constant. *Dalechampia scandens* produces a constant number of nine seeds, so that the trade-off is therefore not present at the level of the blossom. Nevertheless, selection could potentially change the total number of seeds a plant produces. This study is among the first to find correlation between seed size and blossom size.

The slopes of the genetic regressions were considerably steeper than the environmental slopes. This strongly suggests that covariation between blossom size and seed size have a very small role in explaining the within-individual variation in seed size, despite that a high proportion of the total variation in seed size is generated at this level. Because the experiment was performed in uniform greenhouse conditions, we expect that variation among individuals should mainly be due to genetic variation. While the slope of the genetic regression was steeper than the phenotypic regression in Tulum, the slopes were similar in Tovar. One explanation for this difference between the two populations could be that blossom size varied

more within individuals in Tulum ($CV^2 = 0.75\%$) than in Tovar ($CV^2 = 0.56\%$). High variation due to environmental effects may mask genetic correlation and generate shallower phenotypic regression across individuals (Cheverud 1988; Waitt & Levin 1998). Because of the high within-individual variation in Tulum, more blossoms would need to be measured to get a more precise estimate of the breeding value of individuals and therefore a proper estimate of the genetic regression slope, compared to Tovar.

Seed size and blossom size are likely to be under different selection pressures, and thus to be decoupled. While blossom size is often under varying selection pressures depending on pollinator abundance (Schemske & Horvitz 1989), seed size optimum should be temporally constant (McGinley *et al.* 1987). The floral traits involved in pollen exchange are functionally integrated (Armbruster *et al.* 2014), and are expected to be under common selection by pollinators (Armbruster *et al.* 2005; Bolstad *et al.* 2010; Pélabon *et al.* 2012; Pérez-Barrales *et al.* 2013). On the other hand, a variety of factors are found to affect optimum seed size, including advantages of large seeds when seedlings grow in an environment with high competition, shading or nutrient deprivation (Leishman & Wright 2000), while seed predators may cause selection for smaller seeds (Gómez & Husband 2004; Fricke & Wright 2016). Under these circumstances, genetic correlations may represent an adaptive constraint. Decoupling of the traits will allow seed size and blossom size to evolve independently of each other when different selection pressures work on the traits in different directions. If seed size is under strong stabilizing selection around the optimum, seed size needs to be decoupled from blossom size in order to achieve canalization. The correlated response of seed size to selection on blossom size found in this study indicates that some of the same genes controlling for blossom size are also involved in seed size regulation. However, the response was low and the genetic correlation between seed size and blossom size may not strongly constrain the independent evolution of both traits. Because of the low effect size, even in uniform greenhouse conditions, it is likely that environmental effects in nature will mask the shallow genetic correlation. Therefore, there may not be selection for further decoupling of the traits.

Alternatively, pleiotropic effect may have been selected for. Although the genetic correlation may be of no biological significance within populations under “normal” selection pressures, a population undergoing large changes in one of the traits, requires the traits to be correlated (for functional reasons). So a blossom of a certain size can produce seeds of a certain size

range, but this correlation is not important in the limited range of phenotypes within populations, while it becomes important when selection causes the population to go over to a new range of phenotypes. This would be the case in for example speciation, which would be in agreement with Primack's hypothesis, where seed size and blossom size should correlate across species (Primack 1987).

The genetic regression of seed size on gland size was shallow compared to the genetic regression of bract size on gland size (data from Bolstad *et al.* (2014)), where the slope was 0.66 % and 0.48 in Tulum and Tovar, respectively. Seed size varied little compared to the floral traits, having a very low CV^2 compared gland size, and to CV^2 reported on bract size and style length by Pélabon *et al.* (2011). Between-population crosses performed on *D. scandens* showed that size of hybrid seeds differed little from the average seed size of the maternal population, supporting that seed size is a genetically canalized and highly maternally controlled trait (Raunsgard 2017).

Although seed size responded slightly to selection on gland area, the genetic and phenotypic regressions are shallow. I therefore conclude that these traits are decoupled, which suggests that Primack's hypothesis may be limited to among population correlations, and not within. The proportionally small change in seed size in response to a change in blossom size, in addition to the low variance in seed size compared to the size of floral structures, suggest that seed size is a genetically canalized trait.

Acknowledgements

First of all: a huge thanks to my main supervisor Christophe for having me as a master's student. Your enthusiasm and guidance regarding my project has been invaluable in this process. Since the first time I was introduced to the *Dalachampia*-group, 3 years ago, you have inspired me and thought me how cool science is. Thanks to my co-supervisor Elena for great inspiration and guidance. Thanks to Øystein Opedal for sharing your knowledge, for commenting on my manuscript and for saying "This looks very good" when I had lost faith. Thanks to Jarle Tufto for essential help with statistics. Thanks to Scott Armbruster and Jonathan Wright for listening to my project and giving me feedback. Thanks to the research group for great discussions. Thanks to Astrid Raunsgard for endless discussions regarding our projects (a great apology to the other people in our office) and motivation till the bitter end. Kristina, Nils and Vilde: Thanks for long lunch breaks with conversations of both great and poor quality, and silent moments. Solveig - cheers to all our common crises, thanks for all moral support. Marthe, thanks for all awesome adventures and everyday life. Thanks to Elise Jacobsen, Julianne Jacobsen and Marit for creating a home together. Thanks to Tyler for commenting on my manuscript. A special thank you to my family for all support. And thanks to Bjørk, the best dog in the world, for forcing me out in the forest.

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Appendix

Table A1. Mean \pm SE seed diameter (mm) and mean gland size ($\sqrt{(GA)}$ in mm) in the up and down selected lines in the last generation (F4), with sample size (number of seeds and glands, and how many individuals they are from).

Pop.	Line	Mean seed dm. \pm SE (mm)	No. ind.	No. of seeds	Mean $\sqrt{(GA)}$ \pm SE (mm)	No. ind.	No. glands
Tulum	Up	4.30 \pm 0.010	30	267	5.53 \pm 0.062	40	58
	Down	4.16 \pm 0.007	30	266	4.68 \pm 0.066	36	58
Tovar	Up	3.36 \pm 0.009	31	276	4.54 \pm 0.025	84	251
	Down	3.24 \pm 0.008	27	234	3.59 \pm 0.024	80	239

Table A2. Mean \pm SE gland size ($\sqrt{(GA)}$) \pm SE (mm) in each generation (gen.) and selection line with number of individuals and glands measured and mean \pm SE gland size ($\sqrt{(GA)}$) (mm) and seed size (mm) among only the selected individuals (Sel. Ind.) in each generation and selection line, with number of individuals and number of seeds measured.

Gen.	Pop.	Line	$\sqrt{(GA)} \pm$ SE (mm)	No. ind.	No. glands	Sel. Ind. $\sqrt{(GA)} \pm$ SE (mm)	Sel. Ind. Seed dm \pm SE (mm)	No. ind.	No. seeds
P	Tulum	Up	5.35 \pm 0.029	100	400	5.95 \pm 0.067	4.17 \pm 0.013	16	136
		Down				4.88 \pm 0.044	4.06 \pm 0.016	16	132
	Tovar	Up	4.30 \pm 0.015	178	703	4.77 \pm 0.043	3.22 \pm 0.009	16	137
		Down				3.92 \pm 0.040	3.31 \pm 0.011	16	133
F1	Tulum	Up	5.43 \pm 0.035	68	204	5.87 \pm 0.031	4.14 \pm 0.017	16	143
		Down	5.06 \pm 0.034	68	204	4.71 \pm 0.051	4.01 \pm 0.012	16	144
	Tovar	Up	4.41 \pm 0.027	64	208	4.71 \pm 0.022	3.30 \pm 0.010	16	144
		Down	3.99 \pm 0.025	64	209	3.72 \pm 0.027	3.19 \pm 0.011	16	143
F2	Tulum	Up	5.34 \pm 0.043	63	184	5.80 \pm 0.060	4.23 \pm 0.018	16	142
		Down	4.75 \pm 0.041	58	171	4.31 \pm 0.042	4.14 \pm 0.013	16	142
	Tovar	Up	4.55 \pm 0.031	59	171	4.89 \pm 0.023	3.36 \pm 0.010	16	116
		Down	3.90 \pm 0.027	65	193	3.62 \pm 0.018	3.17 \pm 0.014	16	109
F3	Tulum	Up	5.12 \pm 0.035	65	195	5.52 \pm 0.033	4.16 \pm 0.013	15	118
		Down	4.35 \pm 0.035	64	190	3.94 \pm 0.052	3.90 \pm 0.012	17	136
	Tovar	Up	4.61 \pm 0.028	64	192	4.89 \pm 0.045	3.28 \pm 0.013	17	139
		Down	3.83 \pm 0.029	61	180	3.50 \pm 0.034	3.14 \pm 0.012	16	131
F4	Tulum	Up	5.60 \pm 0.048	59	174				
		Down	4.75 \pm 0.041	58	162				
	Tovar	Up	4.72 \pm 0.041	58	158				
		Down	3.84 \pm 0.029	64	185				