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# Inter-sexual parental conflict over seed provisioning in a mixed-mating vine

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# Abstract

1. Sexual reproduction creates an arena for inter-sexual parental conflict. When multiple paternity occurs, paternal plants are selected to exploit maternal plants for resources invested in their seeds, at the expense of seeds sired by other pollen donors, while maternal plants are selected to allocate resources equally among seeds. This may result in a coevolutionary arms race between the sexes over maternal investment. This arms race may be mediated by uniparentally expressed genes with opposite effects on seed growth (*kinship genomic imprinting*) or by selfish paternal alleles evolving to escape recognition by maternal growth-suppressing genes (*interlocus contest evolution*). Crossing populations with different mating systems can reveal inter-sexual conflict, either because populations have reached different equilibria in the arms race and/or due to the fixation of different alleles involved in the conflict.
2. To test for the effect of mating system on hybrid seed size, I performed within and between-population crosses among four populations of the mixed-mating vine *Dalechampia scandens* (Euphorbiaceae), that differ in outcrossing rates.
3. When the paternal population was more outcrossed than the maternal population, hybrid seeds became larger than the seed size within the maternal population, and vice versa in the reciprocal cross.
4. The results support kinship genomic imprinting, that genes with antagonistic effects on seed growth have coevolved within populations, but that antagonistic forces acting on seed size are stronger in more outcrossed populations where inter-sexual parental conflict is expected to be more intense.

**Keywords:** offspring size, parent-offspring conflict, intralocus conflict, multiple paternity, imprinting, pollination, *Dalechampia*



# Samandrag

1. Seksuell reproduksjon skapar ein arena for genetisk konflikt mellom foreldre. Når frø på ei morsplante har ulike fedre, er pollendonorar selektert til å utnytte morsplanter for ressursar investert i frøa deira, på bekostning av frø fertilisert av andre pollendonorar. Morsplanter, på den andre sida, er selektert til å fordele ressursar jamt mellom alle sine frø. Dette kan resultere i eit koevolusjonært våpenkappløp mellom kjønna over morsplantas investering i frø. Våpenkappløpet kan utarte seg gjennom genomisk imprinting, der uttrykket av gener med motsatt effekt på frøvekst avhenger av om genkopien er nedarva frå mor eller far. Alternativt kan egoistiske genkopiar nedarva frå far evolvere til å unngå deteksjon av veksthemmande genkopiar frå mor. Hybridisering mellom populasjonar med ulike parringssystem kan avsløre antagonistisk koevolusjon, anten fordi populasjonar har nådd ulike stadier i våpenkappløpet eller fordi ulike genvariantar involvert i konflikten har gått til fiksering.
2. For å teste for effekten av parringssystem på hybrid frøstørrelse, utførte eg kryss mellom og innad fire populasjonar av *Dalechampia scandens* (Euphorbiaceae). Denne arten kan både fertilisere seg sjølv (sjølvfertilisering) og bli fertilisert av andre plantar (kryssfertilisering), og raten av kryssfertilisering varierer mellom populasjonar.
3. Når farspopulasjonen hadde ei høgare kryssfertiliseringsrate enn morspopulasjonen, blei hybride frø større enn gjennomsnittleg frøstørrelse i morspopulasjonen, og vice versa i den motsette kryssretninga.
4. Resultata støttar genomisk imprinting, at gener med antagonistiske effektar på frøvekst har koevolvert innad i populasjonar, men at antagonistiske krefter på frøvekst er sterkare i populasjonar med høg kryssfertiliseringsrate, der konflikt mellom foreldre er forventa å ha vore sterkare.



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# Introduction

Seed size is a critical life-history trait in plants, with important consequences for dispersal and seedling establishment (Stebbins 1971; Westoby *et al.* 1992; Westoby *et al.* 1996; Vaughton & Ramsey 1998; Henery & Westoby 2001; Westoby *et al.* 2002; Moles *et al.* 2005). Due to the expected increase in individual offspring fitness with offspring size and the energetic trade-off between offspring size and number, we expect an optimum seed size that maximises maternal fitness (Smith & Fretwell 1974). The pollen donor, on the other hand, does not experience the trade-off between offspring size and number, and should be selected to exploit the maternal plant for resources invested in his seeds (Trivers 1974; Westoby & Rice 1982; Queller 1984; Haig & Wilkins 2000; de Jong *et al.* 2005; de Jong & Scott 2007). In outcrossing plant populations, multiple pollen donors commonly sire seeds on the same plant (Ellstrand 1984; Ellstrand & Marshall 1986; Marshall & Ellstrand 1986; Teixeira & Bernasconi 2007), reducing the probability that current or future seeds of a given maternal plant share paternally derived gene copies (Haig & Westoby 1991). Hence, a given pollen donor will maximise his fitness if all maternal resources are invested in his seeds, at the expense of seeds sired by other pollen donors (Haig & Westoby 1989; Haig 1997; Haig 2000). Consequently, selection should favour paternally derived alleles expressed in seeds that increase nutrient demands on the maternal plant (Haig & Westoby 1989; Haig 1997), and maternal mechanisms that ensure an equal allocation of resources among seeds, thus preventing seeds from developing beyond the maternal optimum (Rice & Holland 1997; Wilkins & Haig 2001). This may result in a coevolutionary arms race between the sexes over seed provisioning (Parker & Macnair 1979; Rice & Holland 1997; Wilkins & Haig 2001; Chapman 2006).

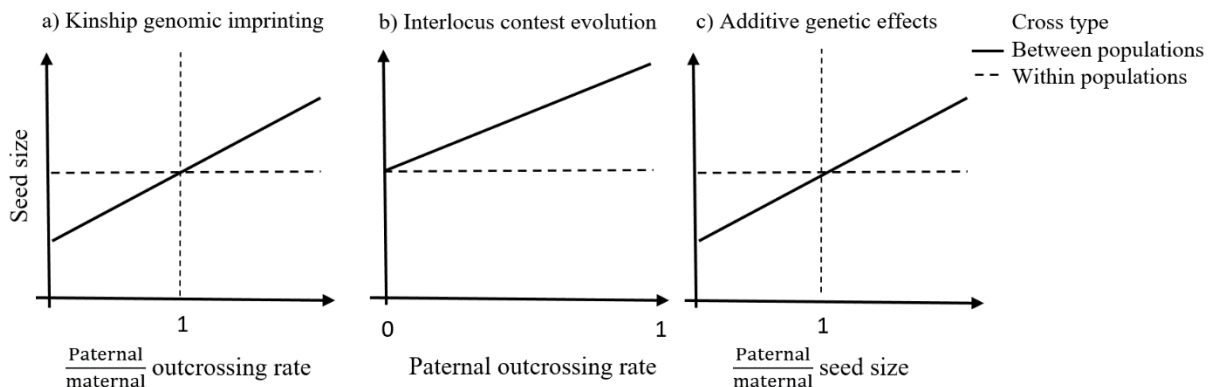
Because seeds acquire all their resources from the maternal plant, the maternal plant is expected to have a strong influence on seed development. Accordingly, substantial maternal effects on seed size are commonly observed (Roach & Wulff 1987; Biere 1991; Platenkamp & Shaw 1993; Byers *et al.* 1997; Lemontey *et al.* 2000), while effects of the pollen donor are generally small (Pittman & Levin 1989; Schwaegerle & Levin 1990; Fenster 1991; Bañuelos & Obeso 2003; House *et al.* 2010; de Jong *et al.* 2011; Pélabon *et al.* 2015; Pélabon *et al.* 2016). The endosperm, which is the nourishing tissue in the seed, generally contains a double dose of maternal genes for a single dose of paternal genes (Sundaresan 2005). The incorporation of a paternal gene copy into the endosperm, has been interpreted as an adaptive strategy of the pollen

donor to increase resource acquisition by the embryo (Queller 1983), while the double dose of maternal gene copies has been interpreted as a strategy of the maternal plant to increase control over resource provisioning (Westoby & Rice 1982; Haig & Westoby 1989).

Imprinted genes in which alleles have differential expression depending on the parent of origin (Efstratiadis 1994; Haig 1997), are important during the endosperm development of angiosperm seeds (Vinkenoog *et al.* 2003). Two genetic mechanisms have been suggested to mediate the conflict over seed provisioning through imprinted genes. According to Haig's *kinship model of genomic imprinting*, loci promoting seed growth should be expressed in the seed when paternally derived, and silenced when maternally derived. Similarly, alleles at growth-suppressing loci should be expressed when maternally derived and silenced when paternally derived (Haig & Westoby 1989; Haig 1997; Haig 2000; Haig & Wilkins 2000; Wilkins & Haig 2001). In its simplest form, the kinship model of genomic imprinting posits that seed size is determined by the additive effects of growth-suppressing and growth-promoting genes, and that antagonistic coevolution between the sexes can lead to an escalation of the number and strength of these genes (Wilkins & Haig 2003). However, antagonistic coevolution does not need to be mediated only by changes in the dosage of gene products (McVean & Hurst 1997). Genes in maternal tissues surrounding the developing seeds, or maternally expressed alleles in seeds may have evolved to recognise and suppress the effects of specific paternally expressed growth-promoting alleles in the seed, a system described by *the interlocus contest model of inter-sexual conflict* (Rice & Holland 1997; Chapman *et al.* 2003; Chapman 2006). Interlocus contest evolution would result in dynamics analogous to host-parasite coevolution (Tellier & Brown 2007), in which growth-promoting genes evolve to escape recognition, and recognition genes evolve to recognise new forms of growth-promoting alleles (Willi 2013).

In both cases considered above, the intensity of inter-sexual parental conflict over seed provisioning is expected to depend on the mating system of the population (Kondoh *et al.* 2000; Brandvain & Haig 2005). Populations with a history of high outcrossing rates, and high frequencies of multiple paternity, should have experienced stronger inter-sexual conflict, and therefore stronger selection for both growth-promoting paternal alleles and counteracting maternal alleles, compared to populations with a history of mostly self-fertilisation (*selfing*) (Kondoh *et al.* 2000; Brandvain & Haig 2005). Within any one population, however, paternal actions to increase seed size and maternal countermeasures are expected to result in some compromise over evolutionary time-scales (Kondoh *et al.* 2000; Brandvain & Haig 2005).

Hybridisation among populations with different mating systems can reveal antagonistic coevolution either because populations have reached different equilibria in the coevolutionary arms race, or because different alleles involved in the conflict have gone to fixation in different populations (Parker & Partridge 1998; Kondoh *et al.* 2000; Andrés & Arnqvist 2001; Brandvain & Haig 2005). In hybrid crosses, alleles from more outcrossed populations are expected to ‘overpower’ alleles from less outcrossed populations (Dawson 1965; Vrana *et al.* 1998; Kondoh *et al.* 2000; Brandvain & Haig 2005), a mechanism termed the ‘weak inbreeder/strong outbreeder’ by Brandvain and Haig (2005). The kinship model of genomic imprinting predicts complementary seed sizes in reciprocal crosses (Haig & Westoby 1991). Linking this to the weak inbreeder/strong outbreeder hypothesis, it then follows that when a more outcrossed maternal population is crossed with a less outcrossed paternal population, hybrid seeds should become smaller than the average seed size within the maternal population (Fig. 1a). This is because the growth-suppressing maternal alleles will have stronger effects than the growth-promoting paternal alleles of the less outcrossed population. Conversely, when a more outcrossed paternal population is crossed with a less outcrossed maternal population, the hybrid seeds should become larger than the average seed size within that maternal population, because the growth-promoting paternal alleles will have stronger effects than the growth-suppressing maternal alleles (Fig. 1a).



**Figure 1.** Predicted outcome on hybrid seed size (solid lines) relative to within-population seed size (horizontal dashed lines) under different genetic scenarios. With kinship genomic imprinting (a) hybrid seed size is predicted to become larger if the paternal population is more outcrossed than the maternal population (to the right of the vertical line), and smaller if the paternal population is less outcrossed. With interlocus contest evolution (b) hybrid seeds are predicted to become larger, and the increase proportional to the outcrossing rate of the paternal population. With additive genetic effects (c) hybrid seed size is predicted to become larger if the paternal population has a larger mean seed size than the maternal population, and smaller if the paternal population has a smaller mean seed size.

Under interlocus contest evolution, maternal or maternally derived alleles may have evolved to recognise and regulate the effects on seed size of paternally derived alleles specific for the population (Rice & Holland 1997; Willi 2013). If different growth-promoting alleles have gone to fixation in different populations, hybridisation could lead to the failure of maternal recognition and seed size regulation when exposed to novel paternally derived alleles (Parker & Partridge 1998; Willi 2013). Therefore, if the pollen donor comes from an outcrossing population, hybrid seeds should then become larger than the normal average seed size within the maternal population, and the magnitude of the increase should depend on the outcrossing rate of the paternal population (Fig. 1b). If the pollen donor comes from a historically completely selfing population, hybrid seed size should remain unchanged from the average seed size within the maternal population, because selection for selfish paternal alleles is not expected in completely selfing populations (Brandvain & Haig 2005).

At least two additional genetic mechanisms may affect seed size during interpopulation hybridisation. First, increased hybrid seed size may result from heterosis, that is, hybrid vigour due to inbreeding depression in the maternal population (Charlesworth & Charlesworth 1987; Lippman & Zamir 2007). Contrary to the predictions from inter-sexual conflict presented above, however, this effect should be independent of the outcrossing rate of the paternal population.

Second, despite the low level of additive genetic variance commonly reported for seed size (e.g. Schwaegerle & Levin 1990; Pélabon *et al.* 2015), some studies suggest that seed size may follow a simple additive genetic inheritance pattern (Lloyd 1968; Alonso-Blanco *et al.* 1999). Depending on local conditions, populations are expected to have different seed size optima and low within-population variation (Smith & Fretwell 1974). Under additive genetic effects, the seed size resulting from hybrid crosses should be intermediate between the seed sizes of the parental populations (Conner & Hartl 2004). If the pollen donor comes from a population with larger seeds, hybrid seeds should become larger than the average seed size within the maternal population, and vice versa with pollen donors from a population with smaller seeds (Fig. 1c). While genes expressed by the embryo should produce a purely additive genetic effect, genes expressed by the endosperm may differ from the purely additive model due to the triploidy of the endosperm. Because the endosperm is dominated by maternal gene copies, hybrid seed size may be expected to be closer to the seed size of the maternal population.

The aim of this study was to test for inter-sexual parental conflict over maternal investment in seed size, and to assess the importance of the mating system in the evolution and resolution of this conflict. I considered predictions of two models of inter-sexual conflict (Fig. 1a and b), and compared these with a simpler additive genetic model (Fig. c), by performing within- and between-population crosses among individuals from wild populations of the mixed-mating vine *Dalechampia scandens*. Populations of this species vary in outcrossing rates and average seed size making this species particularly suitable for testing my predictions. Furthermore, the low variation in the number of seeds produced per fruit, allowed me to control for a potential trade-off between seed size and number.

## Methods

### STUDY SPECIES AND POPULATIONS

*Dalechampia scandens* L. (Euphorbiaceae) is a mixed-mating, perennial vine with a distribution ranging from Mexico to Argentina (Armbruster 1985). Male and female unisexual flowers are aggregated into functionally bisexual inflorescences (*blossoms*) composed of ten male flowers clustered above three female flowers (Webster & Webster 1972). Each female flower has three ovules, so that a blossom can produce up to nine seeds (Webster & Webster 1972; Bolstad *et al.* 2014). A gland situated above the male flowers produces resin that functions as a pollinator reward, attracting apid and megachilid bees that use the resin for nest building (Armbruster 1984; Armbruster 1985). Two petaloid bracts subtend the flowers and function as a signal to attract pollinators (Pérez-Barrales *et al.* 2013). Outcrossing rates in natural populations range from 0.16 to 0.49 (Opedal *et al.* 2016a). The receptive period of the blossom starts with a female phase, during which the male flowers are closed and the stigmas are receptive to pollinators. The bisexual phase starts after 2-3 days when the first male flower opens. During this phase, pollen can fall from the anthers onto the stigmas, and self-fertilisation can occur (Armbruster 1985; Opedal *et al.* 2016b). The rate of autofertility (seed set in the absence of pollinators) depends on the distance between anthers and stigmas (*herkogamy*), a highly evolvable trait that varies among populations (Armbruster 1985; Hansen *et al.* 2003; Opedal *et al.* 2015; Opedal *et al.* 2016a). Populations with high autofertility rates tend to have low outcrossing rates, and thus herkogamy is often used as a proxy for variation in mating system (i.e. outcrossing rate) (Armbruster 1988; Moeller 2006; Herlihy & Eckert 2007; Dart *et al.* 2012; Opedal *et al.* 2015).

In this study, I used four populations of *D. scandens*, originating from Veracruz and the Yucatán peninsula in Mexico, that differ in average seed size and herkogamy (Table 1; Bolstad *et al.* 2014). The populations are inter-fertile but geographically separated by at least 225 km, so that natural gene flow between these populations should be extremely rare (Opedal *et al.* 2017).

**Table 1.** Coordinates, average anther-stigma distance (ASD) and average seed diameter of the four study populations of *D. scandens* used for the crossing experiment.

<b>Population</b>	<b>Coordinates</b>	<b>ASD <math>\pm</math> SE (mm)</b>	<b>Seed dm <math>\pm</math> SE (mm)</b>
Comalcalco (C)	18°21'26" N, 93°20'43" W	2.61 $\pm$ 0.28	3.89 $\pm$ 0.023
Puerto Morelos (PM)	20°51'11" N, 86°53'43" W	2.62 $\pm$ 0.19	4.30 $\pm$ 0.035
Martinez de la Torre (M)	20°05'09" N, 97°01'55" W	2.95 $\pm$ 0.27	4.14 $\pm$ 0.031
Ciudad del Carmen (CC)	18°56'29" N, 91°18'01" W	3.40 $\pm$ 0.16	4.15 $\pm$ 0.043

## EXPERIMENTAL DESIGN

The experimental populations were grown between May and July 2016 from seeds obtained from random within-population crosses of first greenhouse-generation plants. Hence, the experimental individuals used here were second greenhouse-generation plants. Ten plants from each population were used for the crossing experiment. Individuals from the different populations were evenly distributed across two tables in a single room in the greenhouse at the Department of Biology (NTNU) (13hrs light (25°C)/11hrs dark (23°C), watered every day by flooding tables with ca. 5 cm of water). Plants were moved weekly to avoid positional effects.

All four populations were crossed in a complete diallel design, with each population used both as the paternal and maternal population (Table 2). This resulted in four sets of within-population crosses and twelve sets of between-population crosses. For the within-population crosses, all ten plants in the population were crossed with two different plants from the same population (outcrossing) and once with itself (geitonogamous selfing). For the between-population crosses, each population was crossed both as maternal population and as paternal population with each of the three other populations. For each combination of two populations, ten plants from the maternal population were crossed with three different plants from the paternal population. Hence, each maternal and paternal individual was represented three times in the crossing design. Crosses (total  $n = 460$ ) were conducted from August to December 2016.

**Table 2.** Number of seeds produced (and number of crosses) in the 16 cross combinations within (self/outcross) and between the four populations of *D. scandens* (♀: maternal population, ♂: paternal population).

	♂C	♂CC	♂M	♂PM
♀C	80 (11)/199 (15)	248 (29)	220 (28)	244 (30)
♀CC	230 (28)	82 (10)/174 (20)	255 (30)	258 (30)
♀M	232 (26)	234 (28)	88 (10)/180 (21)	261 (30)
♀PM	216 (28)	235 (29)	216 (27)	73 (9)/169 (21)

Blossoms were emasculated and hand-pollinated during the female phase. An ample amount of pollen from a freshly dehisced male flower was deposited on the stigmas to ensure full seed sets. The same pollen donor was used in several crosses, as long as there were sufficient amounts of pollen (up to four crosses). Crosses that failed were recorded, and repeated for as long as time allowed. The sequence of performing the different cross combinations was random to avoid possible confounding factors associated with the timing of pollination and uncontrolled variation in the greenhouse environment. To control for the effect of blossom size on seed size, the peduncle diameter, which correlates with blossom size (Pélabon *et al.* 2015), was measured with digital callipers (0.01 mm precision). Hand-pollinated blossoms were enclosed in empty tea bags, so that seeds could be collected following explosive dehiscence. Plants were observed daily throughout the experiment.

## SEED MEASUREMENTS

The number of seeds produced per blossom was counted (*seed set*), and the diameter of each individual seed was measured (*seed size*) with digital callipers (0.01 mm precision). One repeated measurement was conducted for one seed per seed set, to estimate measurement error. The repeatability was high ( $r^2 = 0.99$ ,  $n = 447$  repeated measurements) indicating a very low measurement error. Seeds from different cross combinations were measured in random order. I weighed the mass of seed sets to estimate the allometric relationship between seed diameter and average seed mass. Seed diameter and mass were strongly correlated ( $r^2 = 0.76$ ,  $n = 430$  seed sets), but because seed diameter is less prone to vary with time due to water loss, it represents a more reliable measure of seed size.

## STATISTICAL ANALYSES

### *Incompatibility between populations*

To assess possible incompatibilities between populations, I calculated the percentage of crosses that failed to produce seeds, and the percentage of aborted seeds for each cross combination. Because each blossom of *D. scandens* can produce a maximum of nine seeds, I subtracted the number of viable seeds from nine, to obtain the number of aborted seeds (non-developed and abnormally small seeds). I tested for differences among cross combinations for each maternal population in the proportion of crosses that failed to produce seeds and the proportion of aborted seeds using Fisher's exact test. Finally, I calculated the average number ( $\pm$  SE) of viable seeds produced per blossom for each cross combination. In cases where I had replicated a specific cross between two individuals, I only included data from the first cross performed. In the following analyses on seed size, I included the crosses that produced the highest number of seeds, in cases of replication.

### *Effect of paternal population on seed size*

To test whether hybrid seed size depended on paternal population, I fitted separate linear mixed-effects models for each maternal population, with seed diameter as response variable and paternal population identity as predictor variable. Paternal population was treated as a dummy variable with five levels: pollen from the same plant (selfing), pollen from another individual in the same population (within-population outcross), and pollen from each of the three other populations (between-population cross). Effects of peduncle diameter and number of seeds per blossom on seed size were population-specific (Table A2, Appendix). Therefore, both variables were centred on the mean of the maternal population and included as covariates. Number of seeds per blossom was also allowed to interact with paternal population identity to account for a potential cross-specific size-number trade-off. Blossom identity nested within maternal plant identity, and paternal plant identity were set as random factors. From these models, I estimated the average seed size produced in each cross combination (Table A2, Appendix).



### *Effects of mating system on hybrid seed size*

To test for parental conflict over resource provisioning to seeds, I assessed whether the mating system of the populations involved explained variation in hybrid seed size. The maternal population was expected to have the strongest influence on hybrid seed size. Therefore, I expressed hybrid seed size as a percentage deviation from the average seed size produced by within-population outcrossing in the maternal population (*hybrid relative seed size*). Averages for each maternal population, were obtained from the models described above (see Table A2, Appendix).

To test the kinship model of genomic imprinting (Fig. 1a), I defined a mating system index that quantifies the relative outcrossing rate of the two parental populations based on their mean anther-stigma distances (ASD), as:

$$\text{Mating system index} = \log \left( \frac{\text{Paternal population ASD}}{\text{Maternal population ASD}} \right),$$

where log is the natural logarithm. Positive values of the index indicate that the paternal population is more outcrossed than the maternal population, and negative values indicate the opposite. I then fitted a linear mixed-effects model using hybrid relative seed size as a response variable, and the mating system index as a predictor variable. To account for dependency among observations, I set the random factors to be blossom identity nested within maternal plant identity, nested within maternal population identity, and paternal plant identity nested within paternal population identity. Mean-centred peduncle diameter in interaction with maternal population identity, and mean-centred seed number in interaction with maternal and paternal population identity, were included as covariates.

To test the interlocus contest model (Fig. 1b), I fitted two different linear mixed-effects models. If hybridisation affects the maternal control over seed size due to failed recognition of novel paternal allele products, seeds of crosses between populations are expected to be larger than seeds produced by within-population crosses. To test this prediction, I fitted a linear mixed-effects model using hybrid relative seed size as a response variable and cross type (between and within) as a predictor variable. To separate a potential effect of larger hybrids from an heterosis effect, I tested whether hybrid seed size depended on the mating system of the paternal population by fitting a linear mixed-effects model using hybrid relative seed size as the response

variable, and the anther-stigma distance of the paternal population as a predictor variable. For both models, covariates and random effects were the same as specified above.

#### *Additive genetic effects on seed size*

To test whether seed size follows a simple additive genetic inheritance pattern (Fig. 1c), I took advantage of the among-population variation in seed size, predicting that average seed size within paternal populations would influence hybrid relative seed size. I defined a parental seed size index that quantifies the relative average seed size of the two parental populations (excl. selfed seeds) as:

$$\text{Parental seed size index} = \log \left( \frac{\text{Paternal population seed diameter}}{\text{Maternal population seed diameter}} \right).$$

I then fitted a linear mixed-effects model with hybrid relative seed size as the response variable and the parental seed size index as the predictor variable. Covariates and random effects were the same as specified above. All statistical analyses were conducted in *R* version 3.3.3 (R Core Team 2017), and linear mixed-effects models were fitted using the *lme4*-package (Bates *et al.* 2015).

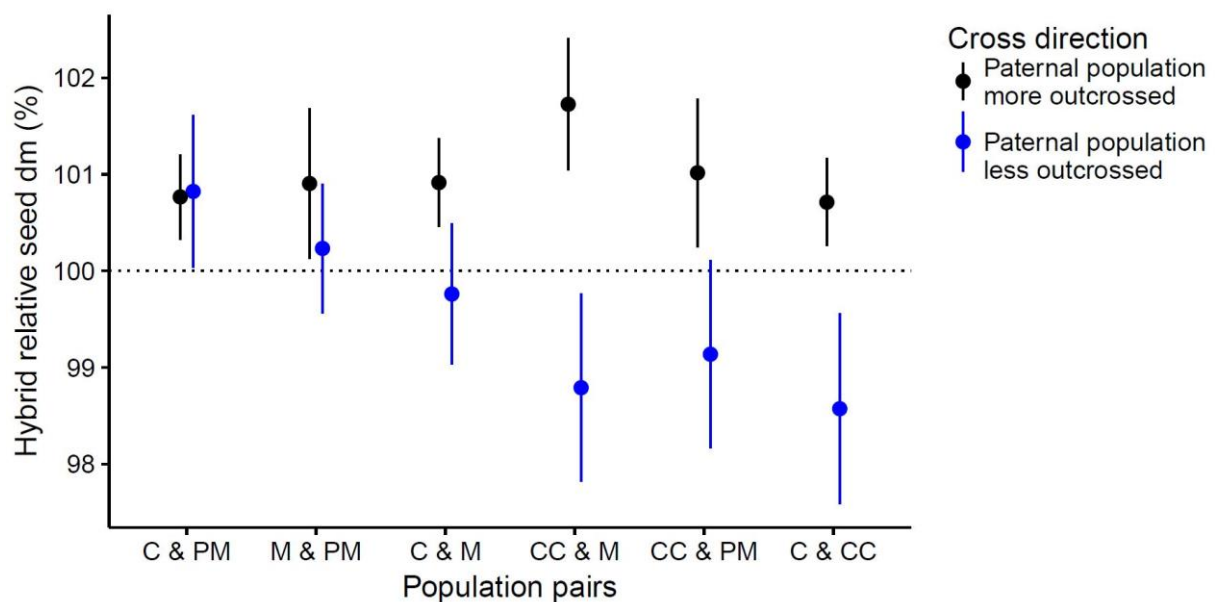
## Results

#### *Incompatibility between populations*

There was no indication of incompatibility between populations (Table A1, Appendix). The percentage of crosses that failed to produce seeds was low (range = 0-10%), and did not differ among cross combinations for any given maternal population (Fisher's exact test; C:  $p = 0.17$ , CC:  $p = 1$ , M:  $p = 0.26$ , PM:  $p = 0.5$ ). The average number of seeds produced per blossom was high for all cross combinations (range = 7.4-8.9 seeds). The percentage of aborted seeds ranged from 3.1% - 17.4%, and did not differ among cross combinations for most maternal populations (Fisher's exact test; C:  $p = 0.59$ , CC = 0.22, PM:  $p = 0.37$ ), but a significant difference was detected for maternal population M (Fisher's exact test;  $p < 0.001$ ). However, the highest percentage of aborted seeds was in within-population crosses (12%), as compared to between-population crosses (range = 1.3-8.8%; Table A1, Appendix). Hence, this difference cannot be explained by incompatibility.

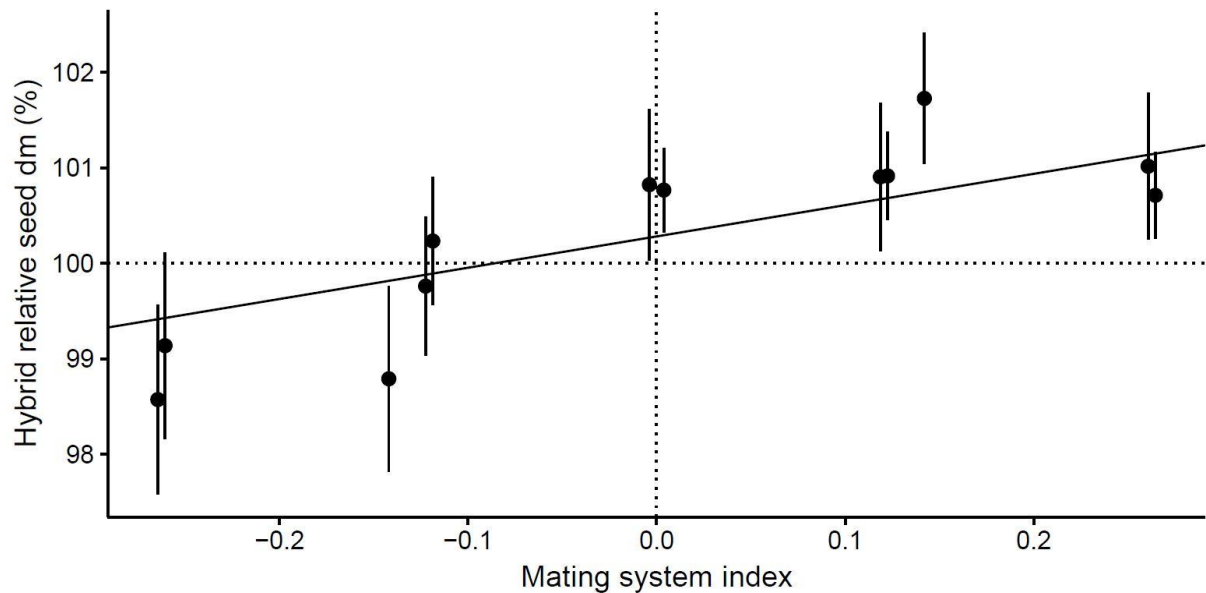
### *Effect of hybridisation on seed size – evidence for inter-sexual parental conflict*

Deviations in hybrid relative seed size (percentage deviation from the average seed size produced within the maternal population) were small (range = -1.4%-1.7%, Table A2, Appendix). Nevertheless, reciprocal crosses produced, in most cases, complementary relative seed sizes (Fig. 2), as predicted by the kinship model of genomic imprinting. Furthermore, in the cross direction in which the paternal population was inferred to be the most outcrossed, hybrid relative seed size was larger than in the opposite cross direction (Fig. 2), as predicted by the weak inbreeder/strong outbreeder hypothesis.



**Figure 2.** Hybrid seed diameter relative to the average seed diameter within the maternal population (dashed horizontal line), in reciprocal crosses of six population pairs. Black dots: paternal population more outcrossed than maternal population, blue dots: maternal population more outcrossed than paternal population. Population pairs are ordered by increasing difference in outcrossing rate (inferred by average anther-stigma distance (mm)). Means  $\pm$  SE for each hybrid cross combination were obtained from the linear mixed-effects models fitted for each maternal population (Table A2, Appendix). For sample sizes, see Table 2.

Hybrid relative seed size increased by 3.28% ( $\pm 1.19$  SE) per unit increase in the mating system index (Fig. 3; Table A3, Appendix). Moreover, the mating system index consistently explained whether hybrids were smaller or larger than the average seed size within the maternal population (Fig. 3).

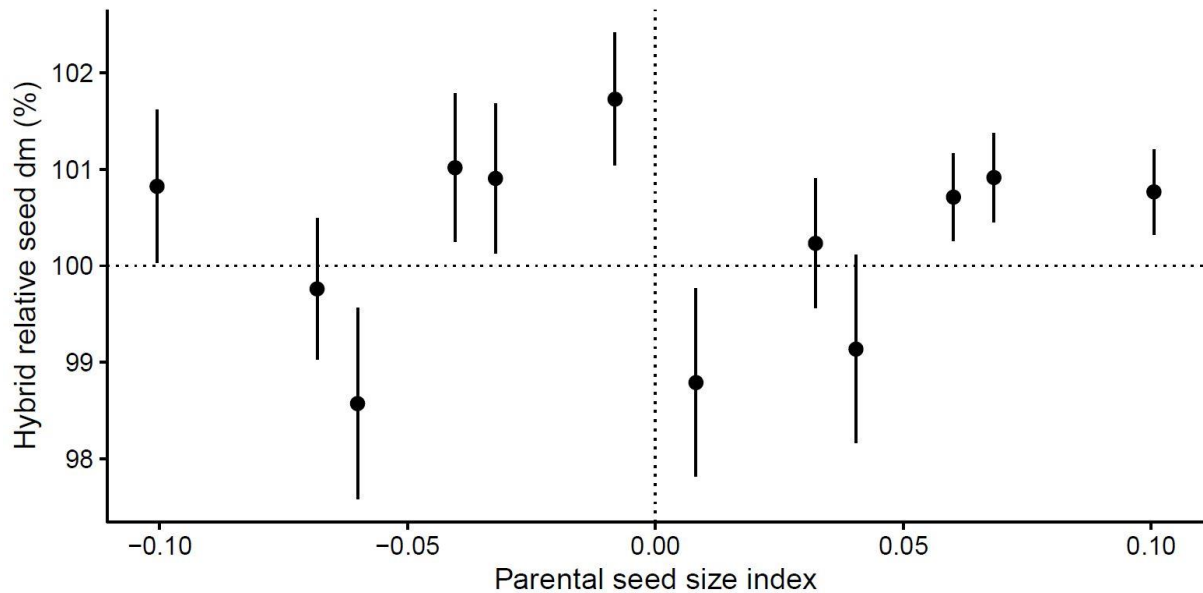


**Figure 3.** Effect of the mating system index,  $\log(\text{paternal population ASD}/\text{maternal population ASD})$  on hybrid relative seed size (hybrid seed diameter as a percentage of the average seed diameter within the maternal population; dashed horizontal line). Positive values of the index: the paternal population more outcrossed than the maternal population. Negative values: maternal population more outcrossed than the paternal population. Each data point represents the mean  $\pm$  SE for each hybrid cross combination, and were obtained from the linear mixed-effects models fitted for each maternal population (Table A2, Appendix). For sample sizes, see Table 2.

Contrary to what was predicted by interlocus contest evolution, between-population crosses did not systematically produce larger seeds than within-population crosses (contrast =  $0.11 \pm 0.30\%$ ,  $t = 0.37$ ,  $p = 0.71$ ; Table A4, Appendix), and hybrid relative seed size did not increase consistently with the inferred outcrossing rate of the paternal population ( $\beta = 0.81 \pm 0.49\%$ ,  $t = 1.67$ ,  $p = 0.10$ ; Fig. A1, Table A4, Appendix).

*Effect of hybridisation on seed size – lack of evidence for additive genetic effects*

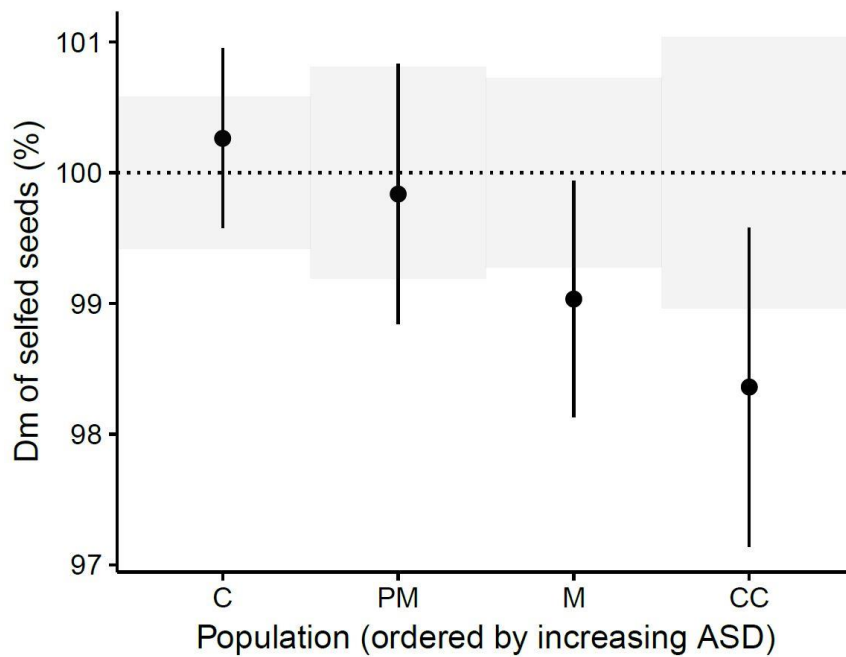
The parental seed size index did not explain variation in hybrid relative seed size ( $\beta = 3.34 \pm 4.12\%$ ,  $t = 0.81$ ,  $p = 0.42$ ; Fig. 4; Table A5, Appendix).



**Figure 4.** Effect of the parental relative seed size,  $\log(\text{paternal population seed diameter}/\text{maternal population seed diameter})$ , on hybrid relative seed size (hybrid seed diameter as a percentage of the average seed diameter within the maternal population; dotted horizontal line). Positive values of the index: paternal population had a larger average seed size, negative values: the maternal population had a larger average seed size. Each data point represents the mean  $\pm$  SE for each hybrid cross combination, and were obtained from the linear mixed-effects models fitted for each maternal population (Table A2, Appendix). For sample sizes, see Table 2.

*Effect of selfing on seed size – lack of evidence for inbreeding depression*

There was no statistically significant difference in seed size between seeds produced by selfing and within-population outcrossing in any of the populations (Fig. 5; Table A2, Appendix). Nevertheless, the difference in size between selfed and outcrossed seeds tended to increase with the (inferred) outcrossing rate of the population. This result suggests that selfing has a more negative effect on seed size in populations where outcrossing, inferred by anther-stigma distance, is more frequent.



**Figure 5.** Effect of selfing on hybrid relative seed size (diameter of selfed seeds as a percentage of the average seed diameter of outcrossed seeds within populations; dashed horizontal line) in four populations of *D. scandens*. Shaded areas represent the standard error in seed size for outcrossed seeds in the four populations of *D. scandens*. Means  $\pm$  SE for each cross combination were obtained from the linear mixed-effects models fitted for each maternal population (Table A2, Appendix).

## Discussion

Crosses between natural populations may reveal inter-sexual conflict over seed size when this conflict has reached different equilibria in the parental populations.

In crosses among populations of *D. scandens*, I found that hybrid relative seed size (hybrid seed diameter as a percentage of the average seed diameter within the maternal population) was best explained by the proportional difference in mating system of the paternal population relative to the maternal population. When the paternal population had a higher outcrossing rate (inferred by the level of herkogamy) than the maternal population, hybrid seeds generally became larger than the average seed size within the maternal population, and vice versa, when the maternal population had a higher outcrossing rate than the paternal population (Figs. 2 and 3). These results are consistent with the weak inbreeder/strong outbreeder hypothesis, suggesting that paternal actions to increase seed size are matched by maternal countermeasures within populations, while the more outcrossed population overpowers the less outcrossed population during interpopulation hybridisation (Brandvain & Haig 2005). These results agree with the results of the classic study of Lloyd (1968), who found that the seed mass resulting from crosses between more or less self-compatible lines of two *Leavenworthia* species depended on which parent was the more outcrossed. It has also been demonstrated that crosses between a monogamous and a polyandrous species of the rodent genus *Peromyscus* yield hybrid offspring size consistent with the weak inbreeder/strong outbreeder hypothesis (Dawson 1965), and that these effects are caused by the disruption of imprinted genes in the placenta (Vrana *et al.* 1998; Vrana *et al.* 2001).

The complementary hybrid seed sizes resulting from reciprocal crosses among *D. scandens* populations (Fig. 2) indicates that parental conflict over seed size is mediated by the genetic mechanism described by the kinship model of genomic imprinting (Haig 2000; Wilkins & Haig 2001). Imprinted genes influencing offspring growth are well documented in mammals (Bartolomei & Tilghman 1997), crop plants (Costa *et al.* 2012; Yuan *et al.* 2017), and in the endosperm development of the model organism *Arabidopsis thaliana* (Vinkenoog *et al.* 2003; Spillane *et al.* 2007; Haig 2013; Pires *et al.* 2016). Willi (2013) also found support for kinship genomic imprinting in crosses between wild populations of *Arabidopsis lyrata* varying in outcrossing rates (0.1-1), where reciprocal crosses produced complementary seed sizes. My study, on a mixed-mating vine, shows that crosses between populations with subtle differences

in mating system also produces hybrid seeds that deviate from the within-population equilibrium seed size in the direction predicted by kinship genomic imprinting and the weak inbreeder/strong outbreeder hypothesis (Wilkins & Haig 2001; Brandvain & Haig 2005). This is an important extension, given that more than 40% of plant species exhibit mixed mating systems (Goodwillie *et al.* 2005).

Kinship genomic imprinting cannot be distinguished from heterosis effects when hybrid relative seed size became larger than within-population seed size (Fig. 2). However, heterosis effects cannot explain the general pattern of hybrid relative seed size increasing with the mating system index (Fig. 3), because heterosis effects should be independent of the mating system of the paternal population. Hence, an important contribution of heterosis to my results can be ruled out.

Willi (2013) also reported evidence for interlocus contest evolution in *Arabidopsis lyrata*. A consistent increase in hybrid relative seed size with pollen from another outcrossing population is expected if maternal recognition and seed size regulation fails when exposed to novel growth-promoting alleles. In my results, hybrid relative seed size tended to be larger more often than smaller (Figs. 2 and 3). However, because there was no consistent increase in hybrid relative seed size, I cannot conclude that interlocus contest evolution is important in my study system. Instead, my results suggest that antagonistic coevolution between the sexes over seed size is mediated by an escalation in the dosage of growth-promoting and growth-suppressing gene products, rather than the evolution of growth-promoting genes to escape recognition.

Crosses among nine populations of *D. scandens* (including the four experimental populations in this study) have been conducted previously for other purposes (see Table A6, Appendix, for population characteristics). This data set is, however, strongly unbalanced, with missing data for many cross combinations, and few samples per cross combination ( $n = 635$  seeds from 86 crosses; Table A7, Appendix). Still, when tested graphically, the pattern observed in the main experiment was upheld across this larger sample of populations, with a general increase in hybrid relative seed size with the mating system index (Fig. A2, Appendix). Deviations in hybrid relative seed size were larger in these data (>5%) compared to the experimental data, which could be explained by the broader range in outcrossing rates (inferred by anther-stigma distance; range = 0.33-3.40 mm, up to ~200% difference) among these populations, compared to in the main experiment (range = 2.61-3.40 mm, up to ~30% difference).



The average seed size within the paternal population did not explain variation in hybrid relative seed size (Fig. 4), as shown previously in *A. thaliana* (de Jong *et al.* 2011). This pattern was also observed in the additional data set (Fig. A3, Appendix). This result excludes the possibility that seed size is determined primarily by additive genetic effects, suggesting a more complex inheritance pattern. Hybrid seed size closely matched the seed size produced within the maternal population, and this result confirms the strong maternal effects on seed size observed in previous studies (e.g. Roach & Wulff 1987; Biere 1991; Platenkamp & Shaw 1993; Lemontey *et al.* 2000). Furthermore, it demonstrates that paternal effects on seed size are weak, complementing previous observations of small paternal effects within *D. scandens* populations (Pélabon *et al.* 2015; Pélabon *et al.* 2016).

Small paternal effects on seed size implies a low additive genetic variance, and thus, low evolvability for seed size (Conner & Hartl 2004). Why then, do we observe such large variation in seed size among populations (Table A7, Appendix)? Among-population variation in seed size can reflect adaptations to local conditions (Smith & Fretwell 1974). Large seeds allow higher germination rates, which is beneficial in habitats with a long growing season (de Casas *et al.* 2017). Large seeds can also increase the competitive ability of seedlings in closed, shaded habitats (Foster 1986), and enhance the ability of seedlings to cope with resource deficits (Westoby *et al.* 1996; Kidson & Westoby 2000; Green & Juniper 2004). On the other hand, smaller seeds allow plants to produce a higher seed number (Smith & Fretwell 1974), and can allow for a higher dispersal potential (Foster 1986). Furthermore, small seeds can persist longer in seed banks (Thompson 1987), and can have a higher probability of escaping predation (Gómez & Husband 2004), for example because the seed coat of smaller seeds can be harder to break by predators (Fricke & Wright 2016). The simplest explanation for how seed size can diverge among populations despite low evolvability, is that selection pressures acting on seed size are strong. To fully understand the evolution of seed size in *Dalechampia scandens*, further studies should quantify the selection pressures acting on seed size in natural populations.

Genomic imprinting has been suggested to enhance evolvability, because a subset of alleles may remain in the silenced state for several generations, allowing mutations to accumulate (McGowan & Martin 1997; Beaudet & Jiang 2002). The validity of these models is, however, debated (Wilkins & Haig 2003). Paternally derived alleles that promote seed growth could potentially have stronger effects than what we observe, because their effects are partly masked by the counteracting effects of growth-suppressing genes. If the optimum seed size changes due

to a change in selection pressures, genetic effects that are hidden due to antagonistic forces could be revealed and selected upon. If selection shifts towards a smaller optimum seed size, mutations that increase maternal growth-suppressing effects or decrease paternal growth-promoting effects would be beneficial, and vice versa for a shift towards a larger optimum seed size. These alleles would then spread in the population, and the antagonistic forces would evolve towards an equilibrium around the new optimum seed size. Imprinting has been reported in molecular studies to be a rapidly evolving phenomenon in plants (Hatorangan *et al.* 2016) and in mammals (Vrana *et al.* 1998). My results, showing that subtle differences in mating system influence hybrid relative seed size, give further support that genes involved in inter-sexual conflict over seed size can evolve rapidly.

This study indicates that uniparentally expressed genes with opposite effects on seed growth have coevolved within populations of *Dalechampia scandens* as a response to inter-sexual parental conflict. The effect of subtle differences in mating system on hybrid seed size, suggests that genes involved in parental conflict may evolve rapidly in response to changes in mating system. Most studies on inter-sexual parental conflict over seed size have focused on the model organism *Arabidopsis thaliana* (Vinkenoog *et al.* 2003), crop plants (Costa *et al.* 2012; Yuan *et al.* 2017) or populations with extreme differences in mating system (Willi, 2013). To understand how inter-sexual conflict evolves, it is, however, important to conduct studies on populations that represent natural variation in mating system.

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## Appendix

**Table A1.** Percentage of crosses that failed to produce seeds, percentage of aborted/abnormal seeds and average number of viable seeds ( $\pm$  SE) produced per blossom in the first series of crosses performed among and within four populations of *D. scandens* ( $n = 480$  crosses).

<b>Maternal population</b>	<b>Paternal population</b>	<b>Failed crosses (%)</b>	<b>Aborted/abnormal seeds (%)</b>	<b>Avg. number of viable seeds per blossom <math>\pm</math> SE</b>
C	C outcross	6	17.4	$7.44 \pm 0.47$
	C self	10	13.6	$7.78 \pm 0.28$
	PM	0	10.7	$8.03 \pm 0.31$
	M	3	12.7	$7.86 \pm 0.32$
	CC	1	8.6	$8.22 \pm 0.37$
PM	PM outcross	5	11.1	$8.00 \pm 0.48$
	PM self	0	8.9	$8.20 \pm 0.51$
	C	0	15.9	$7.57 \pm 0.37$
	M	10	13.2	$7.82 \pm 0.43$
	CC	3	11.1	$8.00 \pm 0.31$
M	M outcross	0	12.1	$8.35 \pm 0.32$
	M self	10	12.4	$8.89 \pm 0.11$
	C	7	1.33	$8.88 \pm 0.07$
	PM	3	3.1	$8.72 \pm 0.10$
	CC	0	8.8	$8.21 \pm 0.35$
CC	CC outcross	0	4.4	$8.60 \pm 0.17$
	CC self	0	8.9	$8.20 \pm 0.44$
	C	0	8.7	$8.21 \pm 0.23$
	PM	0	4.8	$8.57 \pm 0.16$
	M	0	5.6	$8.50 \pm 0.18$

**Table A2.** Effect of paternal population (C, CC, M, PM) on seed diameter in four populations (pop) of *D. scandens*. Within-population outcrossed seeds were set as intercept. Covariates; peduncle diameter and seed set (number of seeds per blossom) were centred on the mean of the maternal population. Blossom nested within maternal plant identity, and paternal plant identity were set as random factors.

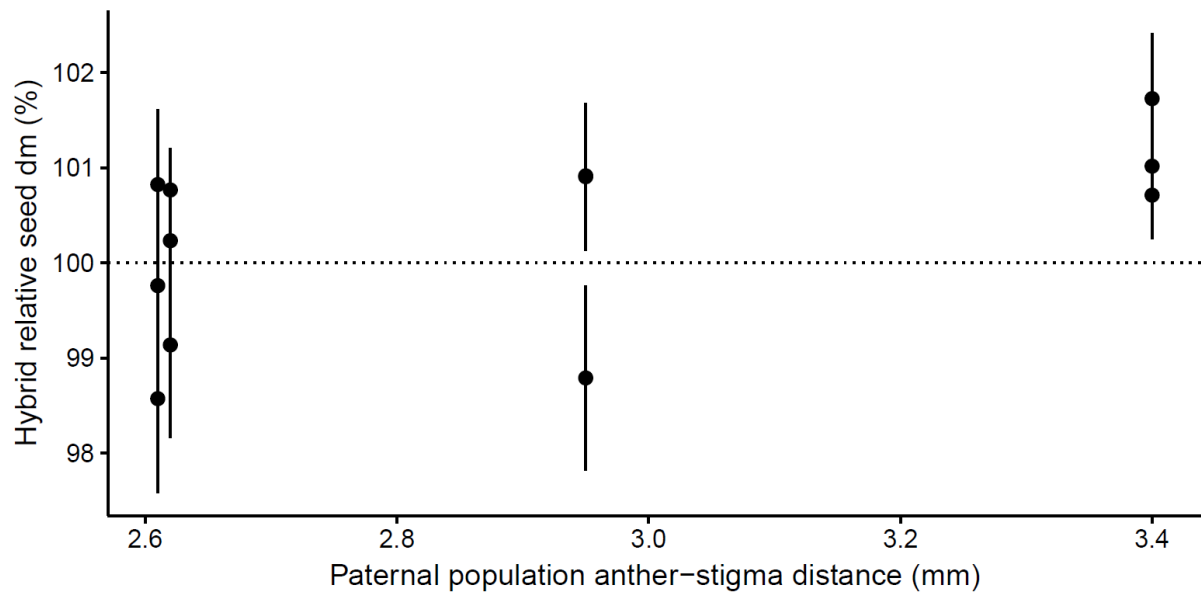
Pop	Parameter	Estimate ± SE (mm)	Estimate ± SE (%)	<i>t</i>	<i>p</i>
C	C outcross (intercept)	3.893 ± 0.023	100 ± 0.582	171	<0.001 ***
	C self	0.010 ± 0.031	0.263 ± 0.799	0.33	0.74
	PM	0.030 ± 0.027	0.766 ± 0.690	1.11	0.27
	M	0.036 ± 0.028	0.915 ± 0.710	1.29	0.20
	CC	0.028 ± 0.028	0.711 ± 0.706	1.01	0.32
	Peduncle	0.112 ± 0.063	2.867 ± 1.612	1.78	0.08
	Seed set	-0.019 ± 0.016	-0.488 ± 0.410	-1.19	0.24
	Seed set × C self	0.010 ± 0.020	0.250 ± 0.516	0.88	0.63
	Seed set × PM	0.028 ± 0.018	0.712 ± 0.563	1.54	0.13
	Seed set × M	0.052 ± 0.018	1.328 ± 0.466	2.85	0.01 **
	Seed set × CC	0.021 ± 0.024	0.549 ± 0.626	0.88	0.38
PM	PM outcross (intercept)	4.305 ± 0.035	100 ± 0.810	123	<0.001 ***
	PM self	-0.007 ± 0.039	-0.163 ± 0.913	-0.18	0.86
	C	0.035 ± 0.032	0.823 ± 0.731	1.13	0.27
	M	0.039 ± 0.031	0.905 ± 0.719	1.26	0.22
	CC	0.044 ± 0.031	1.106 ± 0.708	1.44	0.16
	Peduncle	-0.098 ± 0.070	-2.273 ± 1.626	-1.40	0.17
	Seed set	-0.016 ± 0.013	-0.366 ± 0.306	-1.97	0.23
	Seed set × PM self	0.011 ± 0.025	0.244 ± 0.574	0.43	0.67
	Seed set × C	0.013 ± 0.016	0.295 ± 0.366	0.81	0.423
	Seed set × M	0.027 ± 0.017	0.628 ± 0.389	1.62	0.11
	Seed set × CC	-0.009 ± 0.017	-0.220 ± 0.391	-0.56	0.58
M	M outcross (intercept)	4.168 ± 0.030	100 ± 0.723	138	<0.001 ***
	M self	-0.040 ± 0.037	-0.967 ± 0.875	-1.11	0.27
	C	-0.010 ± 0.035	-0.239 ± 0.850	-0.28	0.78
	PM	0.010 ± 0.033	0.233 ± 0.790	0.30	0.77
	CC	0.072 ± 0.034	1.726 ± 0.810	2.13	0.04 *
	Peduncle	0.375 ± 0.062	9.00 ± 1.478	6.09	<0.001 ***
	Seed set	-0.024 ± 0.019	-0.578 ± 0.463	-1.25	0.22
	Seed set × M self	-0.077 ± 0.106	-1.843 ± 2.550	-0.72	0.47
	Seed set × C	0.132 ± 0.076	3.165 ± 1.823	1.74	0.09
	Seed set × PM	0.009 ± 0.044	0.214 ± 1.061	0.20	0.84
	Seed set × CC	0.038 ± 0.022	0.914 ± 0.538	1.70	0.09
CC	CC outcross (intercept)	4.134 ± 0.043	100 ± 1.039	96.3	<0.001 ***
	CC self	-0.068 ± 0.044	-1.640 ± 1.061	-1.55	0.13
	C	-0.059 ± 0.037	-1.427 ± 0.888	-1.61	0.12
	PM	-0.036 ± 0.036	-0.863 ± 0.871	-0.99	0.33
	M	-0.050 ± 0.036	-1.210 ± 0.871	-1.39	0.17
	Peduncle	0.345 ± 0.091	8.336 ± 2.207	3.78	<0.001 ***
	Seed set	0.028 ± 0.060	0.676 ± 1.459	0.46	0.64
	Seed set × CC self	-0.045 ± 0.066	-1.097 ± 1.583	-0.69	0.49
	Seed set × C	-0.027 ± 0.064	-0.654 ± 1.554	-0.42	0.68
	Seed set × M	-0.077 ± 0.065	-1.830 ± 1.579	-1.16	0.25
	Seed set × PM	-0.022 ± 0.067	-0.524 ± 1.613	-0.33	0.75

**Table A3.** Effect of the mating system index,  $\log(\text{paternal population ASD}/\text{maternal population ASD})$ , on hybrid relative seed size (hybrid seed diameter as a percentage of the average seed diameter within the maternal population; see Table A2 for maternal averages). Covariates; peduncle diameter and seed set were centred on the mean of the maternal population. Blossom nested within maternal plant identity, itself nested within maternal population identity, and paternal plant identity nested within paternal population identity were set as random factors.

<b>Model</b>	<b>Parameter</b>	<b>Estimate <math>\pm</math> SE (%)</b>	<b><i>t</i></b>	<b><i>p</i></b>
Kinship	Intercept	100 $\pm$ 0.29	344.7	<0.001 ***
	Mating system index	3.28 $\pm$ 1.193	2.75	<0.01 **
	Peduncle	3.142 $\pm$ 2.447	1.28	0.20
	Seed set	0.509 $\pm$ 0.350	1.46	0.15
	Peduncle $\times$ matpop CC	5.155 $\pm$ 3.186	1.62	0.11
	Peduncle $\times$ matpop M	3.805 $\pm$ 2.979	1.28	0.20
	Peduncle $\times$ matpop PM	- 5.776 $\pm$ 3.014	- 1.92	0.056 .
	Seed set $\times$ matpop CC	- 0.733 $\pm$ 0.363	- 2.02	0.044 *
	Seed set $\times$ matpop M	0.155 $\pm$ 0.416	0.37	0.71
	Seed set $\times$ matpop PM	- 0.579 $\pm$ 0.323	- 1.80	0.074 .
	Seed set $\times$ patpop CC	- 0.466 $\pm$ 0.323	- 1.44	0.15
	Seed set $\times$ patpop M	0.141 $\pm$ 0.296	0.48	0.64
	Seed set $\times$ patpop PM	- 0.298 $\pm$ 0.295	- 0.76	0.45

**Table A4.** Parameter estimates of two models testing the effect of cross type (within and between populations) and the anther-stigma distance (ASD) of the paternal population, respectively, on hybrid relative seed size (hybrid seed diameter as a percentage of the average seed diameter within the maternal population; see Table A2 for maternal averages). In both models, covariates; peduncle diameter and seed set were centred on the mean of the maternal population. Blossom nested within maternal plant identity, itself nested within maternal population identity, and paternal plant identity nested within paternal population identity were set as random factors.

Model	Parameter	Estimate $\pm$ SE (%)	<i>t</i>	<i>p</i>
Interlocus 1	Within populations (intercept)	100 $\pm$ 2.14	46.7	<0.001 ***
	Between populations	0.11 $\pm$ 0.30	0.37	0.71
	Peduncle	2.84 $\pm$ 2.16	1.31	0.19
	Seed set	0.29 $\pm$ 0.27	1.06	0.29
	Peduncle $\times$ matpop CC	4.29 $\pm$ 2.89	1.49	0.14
	Peduncle $\times$ matpop M	3.94 $\pm$ 2.65	1.48	0.14
	Peduncle $\times$ matpop PM	- 5.16 $\pm$ 2.71	- 1.91	0.057 .
	Seed set $\times$ matpop CC	- 0.57 $\pm$ 0.33	- 1.71	0.088 .
	Seed set $\times$ matpop M	- 0.14 $\pm$ 0.33	- 0.43	0.67
	Seed set $\times$ matpop PM	- 0.49 $\pm$ 0.26	- 1.88	0.06 .
	Seed set $\times$ patpop CC	- 0.17 $\pm$ 0.29	- 0.58	0.56
	Seed set $\times$ patpop M	0.15 $\pm$ 0.26	0.58	0.56
	Seed set $\times$ patpop PM	- 0.15 $\pm$ 0.27	- 0.54	0.59
Interlocus 2	Intercept	97.9 $\pm$ 1.48	66.3	<0.001 ***
	ASD paternal population	0.812 $\pm$ 0.49	1.67	0.10
	Peduncle	3.05 $\pm$ 2.45	1.24	0.21
	Seed set	0.526 $\pm$ 0.351	1.50	0.14
	Peduncle $\times$ matpop CC	5.35 $\pm$ 3.19	1.65	0.10
	Peduncle $\times$ matpop M	3.86 $\pm$ 2.97	1.30	0.20
	Peduncle $\times$ matpop PM	- 5.67 $\pm$ 3.02	- 1.88	0.061 .
	Seed set $\times$ matpop CC	- 0.744 $\pm$ 0.363	- 2.05	0.041 *
	Seed set $\times$ matpop M	0.136 $\pm$ 0.417	0.33	0.74
	Seed set $\times$ matpop PM	- 0.584 $\pm$ 0.324	- 1.80	0.072
	Seed set $\times$ patpop CC	- 0.476 $\pm$ 0.324	- 1.47	0.14
	Seed set $\times$ patpop M	0.124 $\pm$ 0.297	0.419	0.68
	Seed set $\times$ patpop PM	- 0.309 $\pm$ 0.395	- 0.782	0.44



**Figure A1.** Effect of mean anther-stigma distance (mm) of the paternal population on hybrid relative seed size (hybrid seed diameter as a percentage of the average seed diameter within the maternal population; dashed horizontal line). Each data point represents the mean  $\pm$  SE for each hybrid cross combination, and are obtained from the linear mixed-effects models fitted for each maternal population (Table A2, Appendix). For sample sizes, see Table 2.

**Table A5.** Effect of the parental seed size index,  $\log(\text{average seed size within the paternal population}/\text{average seed size within the maternal population})$ , on hybrid relative seed size (hybrid seed diameter as a percentage of the average seed diameter within the maternal population; see Table A2 for maternal averages). Covariates; peduncle diameter and seed set were centred on the mean of the maternal population. Blossom nested within maternal plant identity, itself nested within maternal population identity, and paternal plant identity nested within paternal population identity were set as random factors.

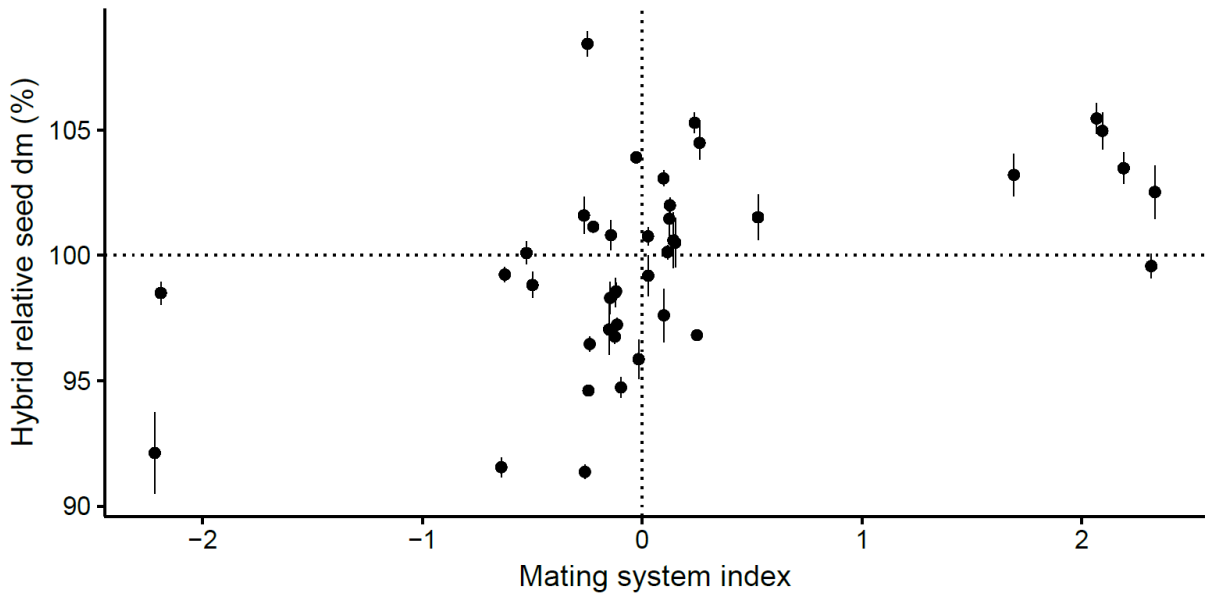
<b>Model</b>	<b>Parameter</b>	<b>Estimate <math>\pm</math> SE (%)</b>	<b><i>t</i></b>	<b><i>p</i></b>
Additive	Intercept	100 $\pm$ 0.487	205	<0.001 ***
	Parental seed size index	3.34 $\pm$ 4.12	0.81	0.42
	Peduncle	2.73 $\pm$ 2.45	1.12	0.27
	Seed set	0.563 $\pm$ 0.351	1.60	0.11
	Peduncle $\times$ matpop CC	5.62 $\pm$ 3.19	1.76	0.079 .
	Peduncle $\times$ matpop M	3.87 $\pm$ 2.98	1.30	0.20
	Peduncle $\times$ matpop PM	- 5.30 $\pm$ 3.02	- 1.76	0.080 .
	Seed set $\times$ matpop CC	- 0.78 $\pm$ 0.36	- 2.15	0.033 *
	Seed set $\times$ matpop M	0.089 $\pm$ 0.42	0.22	0.83
	Seed set $\times$ matpop PM	- 0.60 $\pm$ 0.32	- 1.86	0.064 .
	Seed set $\times$ patpop CC	- 0.50 $\pm$ 0.32	- 1.55	0.12
	Seed set $\times$ patpop M	0.11 $\pm$ 0.30	0.36	0.72
	Seed set $\times$ patpop PM	- 0.33 $\pm$ 0.40	- 0.82	0.41

**Table A6.** Coordinates, average anther-stigma distance (ASD) and average seed diameter of the nine populations of *Dalechampia scandens* in the supplementary data.

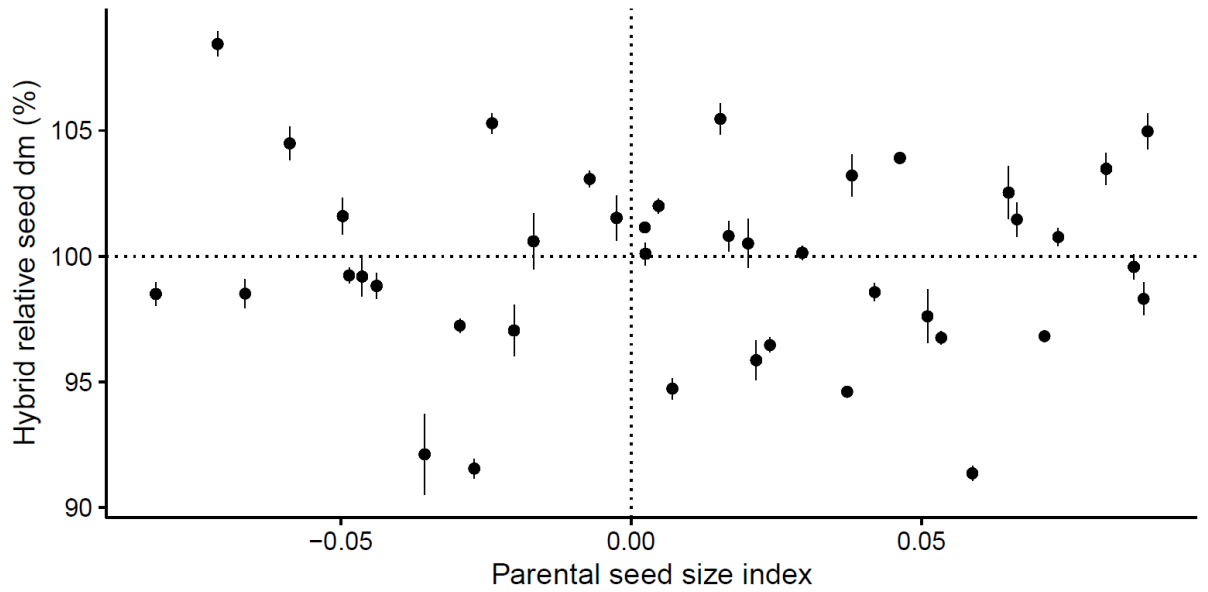
Population	Coordinates	ASD $\pm$ SE (mm)	Seed dm $\pm$ SE (mm)
Bacalar (BA)	18°36'40" N, 88°26'32" W	3.01 $\pm$ 0.31	4.01 $\pm$ 0.027
Comalcalco (C)	18°21'26" N, 93°20'43" W	2.61 $\pm$ 0.28	3.89 $\pm$ 0.023
Cacalchén (CA)	20°58'01" N, 89°13'18" W	1.79 $\pm$ 0.17	4.02 $\pm$ 0.031
Ciudad del Carmen (CC)	18°56'29" N, 91°18'01" W	3.40 $\pm$ 0.16	4.15 $\pm$ 0.043
Graciano Sánchez (GS)	19°08'04" N, 88°30'18" W	2.68 $\pm$ 0.23	4.23 $\pm$ 0.022
La Mancha (LM)	19°37'15" N, 96°28'09" W	0.33 $\pm$ 0.15	3.87 $\pm$ 0.009
Martinez de la Torre (M)	20°05'09" N, 97°01'55" W	2.95 $\pm$ 0.27	4.14 $\pm$ 0.031
Puerto Morelos (PM)	20°51'11" N, 86°53'43" W	2.62 $\pm$ 0.19	4.30 $\pm$ 0.035
Tulum (T)	20°12'26" N, 87°27'04" W	3.34 $\pm$ 0.18	4.22 $\pm$ 0.018

**Table A7.** Number of seeds (and number of crosses) in crosses among the nine populations of *Dalechampia scandens* in the supplementary data. Between-populations crosses:  $n = 635$  seeds (86 crosses). Within-population crosses:  $n = 1427$  seed (176 crosses).

	♂BA	♂C	♂CA	♂CC	♂GS	♂LM	♂M	♂PM	♂T
♀BA	68 (8)	26 (4)	82 (11)	20 (3)	5 (1)	15 (2)	25 (3)	5 (1)	12 (2)
♀C	9 (2)	88 (11)	-	-	8 (1)	-	4 (1)	-	4 (1)
♀CA	39 (5)	-	80 (9)	-	-	-	-	-	-
♀CC	5 (1)	17 (2)	9 (1)	328 (40)	5 (1)	-	6 (1)	9 (1)	23 (3)
♀GS	-	-	-	8 (1)	87 (10)	-	7 (1)	-	-
♀LM	-	7 (1)	7 (1)	24 (3)	9 (1)	408 (50)	37 (5)	-	31 (4)
♀M	15 (2)	4 (1)	22 (3)	30 (4)	8 (1)	37 (5)	86 (10)	6 (1)	15 (2)
♀PM	-	-	-	9 (1)	-	-	-	196 (28)	-
♀T	-	8 (1)	9 (1)	-	6 (1)	-	-	8 (1)	86 (10)



**Figure A2.** Relationship between the mating system index,  $\log(\text{paternal population ASD}/\text{maternal population ASD})$ , and hybrid relative seed size (hybrid seed diameter as a percentage of the average seed diameter within the maternal population; dotted horizontal line) in crosses among nine populations of *D. scandens*. Each datapoint in the Figure represent the mean  $\pm$  SE for each hybrid cross combination, and are calculated from the raw data. For cross combinations and sample sizes see Table A7, Appendix.



**Figure A3.** Relationship between the parental seed size index  $\log(\text{average seed size within paternal population}/\text{average seed size within maternal population})$ , and hybrid relative seed size (hybrid seed diameter as a percentage of the average seed diameter within the maternal population; dotted horizontal line) in crosses among nine populations of *D. scandens*. Each datapoint in the Figure represent the mean  $\pm$  SE for each hybrid cross combination, and are calculated from the raw data. For cross combinations and sample sizes see Table A7, Appendix.