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Original Article

Differences in mating system and predicted parental conflict affect post-pollination reproductive isolation in a flowering plant

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Abstract

Mating system shifts from outcrossing to selfing are frequent in plant evolution. Relative to outcrossing, selfing is associated with reduced parental conflict over seed provisioning, which may result in postzygotic, asymmetric, reproductive isolation in crosses between populations of different mating systems. To test the hypothesis that post-pollination reproductive isolation between populations increases with increasing differences in mating system and predicted parental conflict, we performed a crossing experiment involving all combinations of three self-compatible populations (with low outcrossing rates), and three self-incompatible populations (with high outcrossing rates) of the arctic-alpine herb *Arabis alpina*, assessing fitness-related seed and plant traits of the progeny. Predicted levels of parental conflict ("genome strength") were quantified based on strength of self-incompatibility and estimates of outcrossing rates. Crosses between self-compatible and self-incompatible populations yielded very small seeds of low viability, resulting in strong reproductive isolation. In 14 of 15 reciprocal between-population crosses, seeds were heavier when the paternal plant had the stronger genome, and seed mass differences between cross directions increased with an increased difference in parental conflict. Overall, our results suggest that, when sufficiently large, differences in mating system and hence in expected parental conflict may result in strong post-pollination reproductive barriers contributing to speciation.

Keywords: *Arabis alpina*, breeding system, mating system, parental conflict, reproductive isolation, speciation

Introduction

The shift from outcrossing to selfing is a key evolutionary transition in angiosperms (Barrett, 2002; Sicard & Lenhard, 2011; Wright et al., 2013), often associated with speciation (Cutter, 2019; Foxe et al., 2009; Stebbins, 1957; Wright et al., 2013). Reproductive isolation between outcrossing and selfing lineages may arise from both prezygotic and postzygotic barriers (Briscoe Runquist et al., 2014; Martin & Willis, 2007; Willis & Donohue, 2017). Intrinsic postzygotic barriers are commonly attributed to a gradual accumulation of genetic incompatibilities, as explained by the Bateson–Dobzhansky–Muller model (Bateson, 1909; Dobzhansky, 1936; Muller, 1942; Rieseberg & Blackman, 2010; Sweigart & Willis, 2012). The classic view has been that if such incompatibilities occur between nuclear genes, reproductive isolation should be symmetric. However, reproductive barriers in angiosperms are often asymmetric, with the magnitude of reproductive isolation depending on the direction of the cross (Tiffin et al., 2001; Turelli & Moyle, 2007). This indicates that additional mechanisms may contribute to the build-up of postzygotic reproductive isolation. For example, cytonuclear incompatibilities may cause cytoplasmic male sterility in hybrid plants, thereby resulting in asymmetric reproductive isolation (Caruso et al., 2012; Tiffin et al., 2001). Asymmetries appear to be especially common between closely related species that differ in mating system (Brandvain & Haig, 2005; Cutter, 2019; Lewis & Crowe, 1958; Pickup et al., 2019; Tiffin et al., 2001). Such asymmetries may result from incompatibilities between genes with parent-of-origin specific gene expression, due to parental conflict over resource allocation to offspring (Brandvain & Haig, 2005; Lafon-Placette & Köhler, 2016).

Parental conflict arises when the allocation of resources to seeds differs between the mother and the father (Brandvain & Haig, 2005; Haig & Westoby, 1989, 1991; de Jong & Scott, 2007; Queller, 1983). The maternal plant provides resources to seeds and should thus maximize fitness by distributing resources among all developing seeds (Smith & Fretwell, 1974; Trivers, 1974). In the case of cross-pollination, the paternal plant provides no resources to developing seeds, and should instead be under selection to maximize the (maternal) resource allocation to seeds it sired, but not to seeds sired by other fathers. This conflict is played out in the endosperm,

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the triploid tissue in the seed that receives resources from the maternal plant, and that is crucial for embryo development (Li & Berger, 2012). Mechanistically, the conflict is thought to work through genomic imprinting, whereby different sets of genes are expressed in the endosperm depending on parental origin (Gehring & Satyaki, 2017; Haig & Westoby, 1989; Lafon-Placette & Köhler, 2016; Lafon-Placette et al., 2018). According to parental conflict theory, the paternally inherited genome expresses genes that act to increase resource allocation to the seed, while the maternally inherited genome expresses genes to counteract this, over time resulting in a co-evolutionary arms race (Geist et al., 2019; Kondoh & Higashi, 2000). The conflict should be stronger in highly outcrossing plants with seeds sired by multiple pollen donors, than in predominantly selfing plants, where the same individual provides both the female and male gametes to most seeds (Brandvain & Haig, 2005; Burt & Trivers, 2006; Haig & Westoby, 1991; de Jong & Scott, 2007; Queller, 1983). Consequently, the shift from outcrossing to selfing is expected to change the potential for parental conflict.

Differences in parental conflict (also referred to as maternal/paternal excess, genome strength, endosperm balance number, or effective ploidy (Haig & Westoby, 1991; Johnston et al., 1980)) between outbreeding and selfing lineages is the basis of the weak inbreeder/strong outbreeder (WISO) hypothesis (Brandvain & Haig, 2005). This hypothesis predicts that a cross between a maternal outcrossing (outbreeding) and paternal selfing (inbreeding) plant should lead to a stronger maternal than paternal influence on allocation to seeds (we refer to this as a stronger maternal genome), resulting in limited resource allocation to the endosperm and thus comparatively small seeds. The reciprocal cross with a stronger paternal genome results in increased resource allocation to the endosperm and thus comparatively larger seeds. In this case, however, for plants with a nuclear endosperm development such as Brassicaceae, the endosperm may fail to cellularize correctly, resulting in large but deformed seeds that do not germinate (Brandvain & Haig, 2005; Gehring & Satyaki, 2017; Köhler et al., 2021; Lafon-Placette & Köhler, 2016). Because variation in seed size and viability can affect germination success and subsequent seedling growth (Krannitz et al., 1991; Susko & Lovett-Doust, 2000), parental conflict differences can influence patterns of gene flow and reproductive isolation.

Several studies examining the effects of cross direction on seed size have found support for the WISO hypothesis (Brandvain & Haig, 2005; Lloyd, 1968; Raunsgard et al., 2018; Rebernig et al., 2015; Willi, 2013), whereas effects on germination are more variable, with examples of higher germination in crosses with a stronger maternal (Rebernig et al., 2015) or paternal (Ruhsam et al., 2011; Willis & Donohue, 2017) genome. Often both cross directions produce seeds that are smaller and/or have lower germination compared to intraspecific crosses. This has been observed also in crosses involving rather limited differences in mating system, such as crosses between outcrossing and sometimes self-incompatible lineages (Coughlan et al., 2020; Garner et al., 2016; İltaş et al., 2021; Lafon-Placette et al., 2017; Roth et al., 2018; Sandstedt et al., 2021). Collectively, these previous studies suggest that the importance of parental conflict differences for generating reproductive isolation between lineages should depend on the magnitude of the difference in parental conflict (Haig & Westoby, 1991; Städler et al., 2021). Indeed, small differences in mating system and hence in parental conflict may result in viable seeds differing little in size from parents and between cross directions (Raunsgard et al., 2018), while crosses with larger differences may produce small, poorly germinating seeds in one or both directions (Oneal et al., 2016; Rebernig et al., 2015). Ideally, the effects of parental conflict on seed development should be tested in a species encompassing the whole diversity of mating system from fully outcrossing to selfing. To our knowledge, this has been attempted only in *Arabidopsis lyrata*, with conflicting results for seed size (Gorman et al., 2021; Willi, 2013), and data on germination limited to a single population-pair (İltaş et al., 2021). Hence, more comprehensive investigations are needed to fully examine the importance of the magnitude of parental conflict differences for reproductive isolation.

In this study, we test the hypothesis that post-pollination reproductive isolation between populations increases with increasing differences in mating system and predicted parental conflict using the perennial herb *Arabis alpina* (Brassicaceae). This species is a suitable study system, because it includes populations varying from self-incompatible and outcrossing, to self-compatible and highly selfing (Ansell et al., 2008; Laenen et al., 2018; Petrén et al., 2021; Tedder et al., 2011; Toräng et al., 2017). We use the term mating system to describe variation in outcrossing rate (from outcrossing to selfing), while *breeding system* refers to the presence or absence of a functional self-incompatibility system. We made reciprocal crosses in all possible combinations between three self-compatible and three self-incompatible populations, and examined the effects of cross type (within vs. between breeding systems) and cross direction on seed set, seed size, germination, and offspring size. First, we tested whether seed set, seed size, and germination were lower after crosses between breeding systems compared to crosses between populations of the same breeding system. Second, we asked whether seed size was affected by cross direction in reciprocal crosses as expected if parental conflict over seed provisioning is higher in the more outcrossing populations, and whether an estimate of the magnitude of parental conflict difference could explain the degree of seed mass asymmetry. Third, we examined whether differences in seed size could explain variation in germination success. Finally, we tested whether total reproductive isolation between populations, quantified based on seed set and germination, differed for crosses between and within breeding systems.

Methods

Study species and populations

The arctic-alpine diploid perennial herb *Arabis alpina* occurs in rocky, disturbed habitats in mountain regions in the northern hemisphere (Koch et al., 2006). It likely originated in Asia Minor, with one lineage colonizing Europe and North America and two others spreading through the Middle East and East Africa (Ehrich et al., 2007; Koch et al., 2006). In Europe, populations in central Italy and Greece are self-incompatible and highly outcrossing (Ansell et al., 2008; Laenen et al., 2018; Tedder et al., 2011). Populations from Scandinavia, Spain, and at least parts of the Alps are self-compatible (Ansell et al., 2008; Buehler et al., 2012; Tedder et al., 2011; Toräng et al., 2017). These have evolved smaller flowers that produce less pollen and floral scent, and reduced herkogamy promoting autonomous self-pollination, resulting in lower outcrossing rates, especially in Scandinavia (Petrén et al., 2021; Tedder et al., 2015; Toräng et al., 2017). The origin of self-compatibility in European *A. alpina* is unknown, but the most parsimonious scenario would consist of a single shift from self-incompatibility to self-compatibility, followed by the evolution of autonomous self-pollination in Scandinavian populations (Ehrich et al., 2007; Laenen et al., 2018; Toräng et al., 2017).

To examine the effects of differences in mating system and predicted parental conflict on post-pollination reproductive isolation, we conducted crosses among six European populations of *A. alpina* (Table 1). Three self-incompatible populations from central (It6) and northern (It9) Italy and from Greece (G2) were included in the experiment together with three self-compatible populations from northern Scandinavia (S1), France (Fr2), and Spain (E3). The breeding system of these populations was confirmed by comparing seed sets after manual pollination with self-pollen and pollination with cross-pollen from the same population (Supplementary Figure S1).

To infer the (relative) level of parental conflict in a population or species, previous studies have used various measures, with the most direct one being measures of outcrossing rate (Brandvain & Haig, 2005). For the three self-compatible populations, we used outcrossing rates estimated from the multilocus structure of established plants by Toräng et al. (2017) (E3: $t_m = 0.231$; Fr2: $t_m = 0.139$; and S1: $t_m = 0.0619$; Table 1) to estimate relative levels of parental conflict. While previous research indicates substantially higher outcrossing rates in self-incompatible *A. alpina* populations ($t_m > 0.77$, Tedder et al., 2011), we did not have such data for the self-incompatible populations in our study. For these populations, we instead used the results from controlled self- and cross-pollinations to calculate the index of self-incompatibility (ISI). This index has been found to be positively correlated with the outcrossing rate (Raduski et al., 2012), and we used it as a proxy for the outcrossing rate to estimate the relative level of parental conflict in the self-incompatible populations (It6: $ISI = 0.75$; It9: $ISI = 0.90$; and G2: $ISI = 0.95$; Table 1). This allowed us to rank the predicted genome strength (level of parental conflict) for the populations in our study from lowest to highest: S1, Fr2, E3, It6, It9, and G2. We also quantified the magnitude of parental conflict difference between pairs of populations as *Conflict difference = ISI or* t_m *of higher ranked population – ISI or* t_m *of lower ranked population* (see Supplementary information for calculations of ISI and a justification for its use as a proxy for outcrossing rate).

Experimental setup and conditions

To produce plants for the experimental crosses, we used seeds collected at the sites of populations G2, It6, It9, and S1 in 2014 and 2015, or that were the products of one generation of selfing in the greenhouse (populations Fr2 and E3). Greenhouse-produced seeds were used for the latter two populations because older seeds, collected in the field, had low germinability. In October 2016, seeds from each of the six populations were planted on agar plates and stratified under dark conditions for 1 week at 4°C. Thereafter, agar plates were moved to a growth room with 16 hr daytime conditions at a temperature of 20°C and 150 $\mu Em^{-2}s^{-1}$ light intensity, and an 8 hr, 16°C night. After germination, seedlings were transferred to individual 6 × 6 × 7 cm pots filled with a soil mixture consisting of one part 2–6 mm LECA (Lightweight Expanded Clay Aggregate; Saint-Gobain Byggprodukter, Sollentuna, Sweden) and two parts potting soil ("Yrkes-Plantjord" SW Horto, Hammenhög, Sweden), with a thin topsoil layer of low-nutrient soil ("Plugg och Såjord," SW Horto, Hammenhög, Sweden). After approximately four weeks of growth, plants were vernalized for 12 weeks at conditions maintaining 8 hr days at 4–6°C and 50 $\mu Em^{-2}s^{-1}$, and 16 hr, 4–6°C nights. After vernalization, we moved the plants to a greenhouse with a 16 hr, 18°C day with both artificial and natural lighting, and an 8 hr, 16°C night. Every 2–3 days, pots were automatically watered using water with a low amount of nutrients added (SW Bouyant Rika T 3-1-5 fertilizer, SW Horto, Hammenhög, Sweden). Within 2 weeks, most plants had started flowering and crosses were initiated.

Experimental procedures

We conducted crosses among plants within and between all six populations (Table 1) for a total of 21 possible cross combinations (15 between-population crosses, 6 within-population crosses). For each cross-combination, we used three to six pairs of plants that were cross-pollinated with each other. Individual plants were only crossed with the other plant in the pair. For each plant, we first emasculated three (on rare occasions two or four) buds just before they were about to open to avoid self-pollination. Using sharp-tipped forceps sterilized in ethanol between each pollination event, we hand-pollinated the emasculated flowers with pollen from the other plant in the pair, by gently rubbing detached mature anthers across the stigmas of the recipient plant. This design allowed us to simultaneously perform crosses in both directions for the same individual pair of plants. Pairs of plants were haphazardly

Table 1. Name, location, latitude, longitude, number of individuals used for the crosses, breeding system (SC, self-compatible; SI, self-incompatible), index of self-incompatibility (ISI, see Supplementary information), and outcrossing rate for the six populations of *Arabis alpina*.

Population	Location	Latitude	Longitude	Individuals	Breeding system	ISI	Outcrossing rate[†]
S1	Northern Scandinavia	68° 24' N	18° 19' E	36	SC	0.02	0.0619
E3	Northwest Spain	43° 14' N	05° 56' W	36	SC	0.00	0.231
Fr2	French Alps	44° 57' N	06° 36' E	38	SC	0.03	0.139
It6	Central Italy	41° 50' N	13° 56' E	42	SI	0.75	NA
It9	Northern Italy	44° 05' N	10° 19' E	40	SI	0.90	NA
G2	Northwest Greece	39° 57' N	20° 48' E	36	SI	0.95	NA

[†] Estimated from multilocus structure of established plants in Toräng et al. (2017).

selected from each population with the constraint that, for each cross combination, individuals from different maternal lines were used whenever possible. In total, 228 individual plants were included in the experiment.

We collected fruits at maturation. For each fruit, we counted the number of seeds and determined the total seed mass to the nearest 0.1 mg with a Sartorius analytical scale (Sartorius GmbH, Göttingen, Germany). Mean seed mass was calculated by dividing total mass by the number of seeds. For a subset of fruits, we quantified seed area, as seed mass and area can to some extent vary independently (e.g., Rebernig et al., 2015). Five randomly chosen seeds from the fruits of 166 plants, including at least one plant from each type of cross, were photographed using a digital microscope (Celestron Handheld Digital Microscope Pro, Celestron, Torrance, CA, USA). Images were converted to black and white, and the area of each seed was measured using the "Analyse Particles" function in the software ImageJ (Schneider et al., 2012). For each fruit, we then calculated the mean area per seed. Seeds were stored under dry conditions at room temperature before and after these measurements.

To examine the effects of cross type and direction on seed germination and offspring plant size, we planted a subset of the seeds generated from the crossing experiment in February 2018, approximately 6 months after fruit harvest. Seeds were sown directly on soil in pots prepared in the same way as described above. In the great majority of cases, we planted seeds from one fruit of a maternal plant in each pot. Up to 20 seeds were carefully placed on top of the soil. If less than 20 seeds were available from any one fruit, we used seeds from multiple fruits of the same plant, and if still less than 20 seeds were available, we used all seeds available from that plant (mean = 19.1, range 1–20 seeds planted per pot). In a few cases, no seeds were available, and these crosses had to be excluded. In total, 4,096 seeds were planted from 215 of the 228 maternal plants. After planting, pots were stratified at 6°C in dark conditions for one week before being transferred to a greenhouse with a 16 hr, 20°C day under standard light conditions, and an 8 hr, 16°C night, representing optimal germination conditions for *A. alpina* (Egido et al., 2018). Germination was monitored continuously during the following 2 weeks. At the end of the 2 weeks, new seedlings had stopped emerging, and we counted the number of seedlings in each pot. Following this, we removed all but two seedlings from each pot where multiple seedlings had germinated and transferred one of these to a new pot. Approximately 30 days after pots with seeds were moved to the greenhouse, we measured the rosette diameter of each plant. After an additional 10 days, plants were moved to similar vernalization conditions as the previous generation, where they remained for 13 weeks, after which rosette diameter was measured again. At that time, no plant had begun to flower.

Statistical analyses

We used a set of different models to test the effects of cross type (within vs. between breeding systems) and cross direction (maternal vs. paternal plant stronger) on the number of seeds per fruit, seed mass, germination, rosette size, and level of reproductive isolation. In addition, we examined the effect of seed mass on germination. All analyses were performed in R 4.2.1 (R Core Team, 2022).

First, we examined the effects of cross type and cross direction on the number of seeds per fruit, seed mass, and proportion of seeds germinating. For the number of seeds per fruit and seed mass, we constructed linear mixed models with cross type (three levels: SC × SC, SI × SI, and SC × SI/SI × SC combined; crosses noted as maternal × paternal) and cross direction (maternal vs. paternal plant stronger) as fixed factors and population pair (indicating which two populations were crossed) as a random factor. A subsequent *post hoc* test was performed for pairwise comparisons of cross directions within cross types. Analyses were based on maternal-plant mean values. Models were constructed using the *lme4* package (Bates et al., 2015), significance of fixed factors was tested using the *lmerTest* package (Kuznetsova et al., 2017), and *post hoc* tests were performed with the *emmeans* package (Lenth, 2022). We used the same approach to analyze the proportion of germinated seeds, but instead used a generalized linear mixed model with a beta-binomial error distribution (which accounts for overdispersion (Harrison, 2015)) and a logit link function, using the *glmmTMB* package (Brooks et al., 2017). Because seed mass and seed area were strongly positively correlated ($r = 0.88$, $p < .001$), and effects of cross type and cross direction on the two were similar (Figure 1B, Supplementary Figure S2), analyses of variation in seed size were restricted to seed mass.

Second, we tested for a correlation between the magnitude of parental conflict difference between populations and the magnitude of the seed mass asymmetry (i.e., the magnitude of the effect of cross direction on seed mass). To do so, we used the *Conflict difference* calculated above, and calculated seed size asymmetry as in Coughlan et al. (2020) where *asymmetry = (mean seed mass S × W − mean mass W × S)/(mean seed mass S × W + mean mass W × S)*. *S* and *W* indicate the population having the predicted stronger and weaker genome in the pair, respectively. This measure equals zero if both cross directions produce seeds of equal mass, and is negative if, as predicted, seeds become larger when the paternal population has a stronger genome than the maternal population.

Third, to test the effect of cross type and cross direction on the degree of reproductive isolation, we calculated reproductive isolation (*RI*) for each population-pair × cross-direction combination (15 pairs of crosses between populations × 2 directions for each cross = 30 types of crosses). For germination, this was done following Lowry et al. (2008) as $RI_{Germination} = 1 -$ *[mean germination of inter-population cross]/[mean germination for parental intra-population crosses]*. For the number of seeds per fruit, to not bias the estimation due to potential differences in mean ovule numbers between populations (Stephenson, 1981), reproductive isolation was calculated as $RI_{Seeds} = 1 -$ *[mean seed number of inter-population cross]/[mean seed number for maternal intra-population cross]*. Additionally, we quantified an estimate of total post-pollination reproductive isolation, using the expression $1 - RI_{Total} = (1 - RI_{Seeds}) \times (1 - RI_{Germination})$ (Coyne & Orr, 1989). For measures of *RI*, a value of 0 indicates no reproductive isolation for a specific cross and trait, while a value of 1 indicates complete reproductive isolation. Estimates of *RI* were then used as response variables in two-way ANOVAs with the type of cross (between populations of the same breeding system, SC × SC or SI × SI vs. between populations with different breeding system, SC × SI or SI × SC) and cross direction (maternal vs. paternal plant having a stronger genome) as factors to investigate their effects on reproductive isolation. We also calculated the mean *RI* for both cross directions for each pair of populations and used these values in one-sample

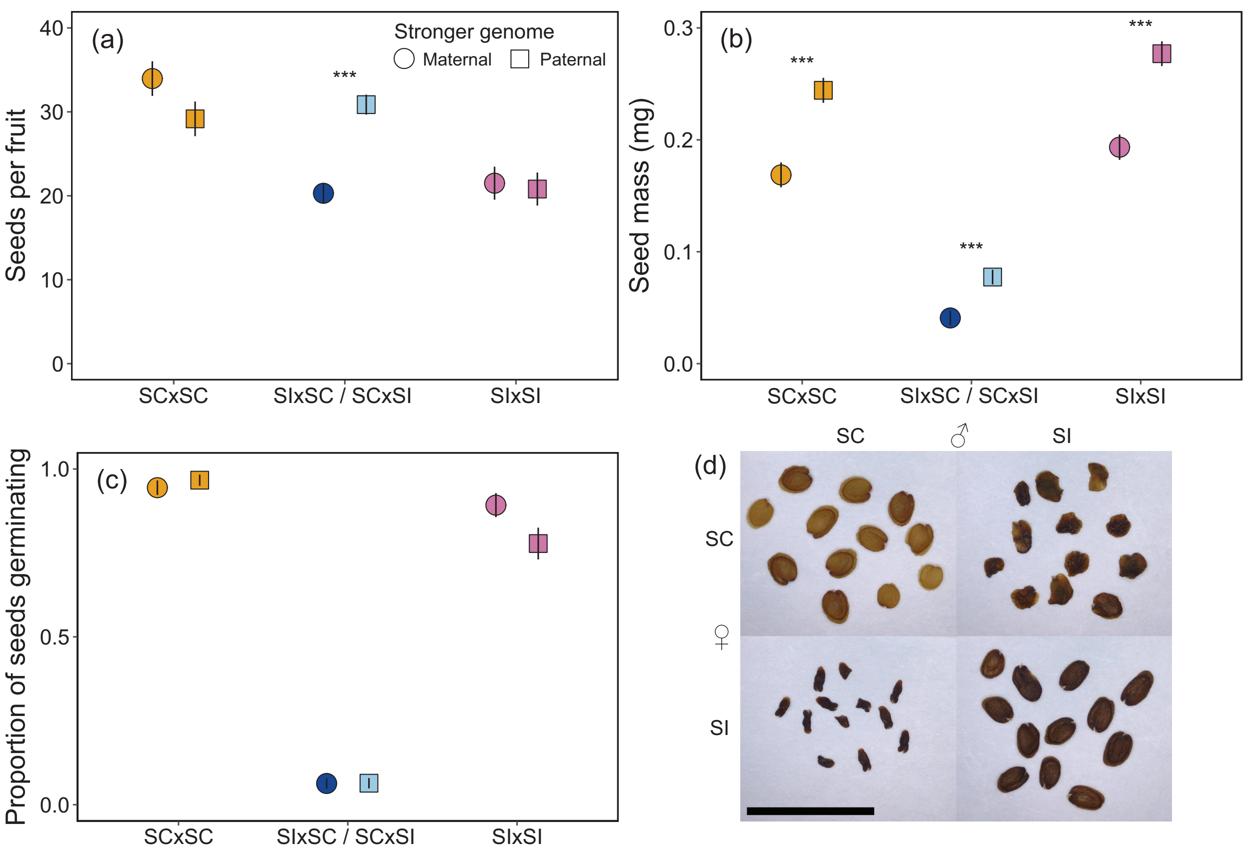

Figure 1. Effects of cross type and cross direction on the number of seeds per fruit (A), seed mass (B), proportion of seeds germinating (C), and seed morphology (D) for crosses between three self-compatible and three self-incompatible *Arabis alpina* populations. Crosses were done between different self-compatible populations (SC × SC, crosses noted as maternal × paternal), between different self-incompatible populations (SI × SI), and between populations of different breeding systems (SI × SC/SC × SI). A circle indicates crosses where the maternal plant was predicted to have a stronger genome, while a square indicates crosses where the paternal plant was predicted to have a stronger genome. This means that for the SI × SC/SC × SI cross type, the circle indicates a SI × SC cross, while the square indicates an SC × SI cross. Points show mean ± SE from the corresponding models. Asterisks indicate significance in comparisons between cross directions within cross types (**p* < .05, ***p* < .01, ****p* < .001). (D) Photo of seeds from crosses between two self-compatible plants (SC × SC; top left), a maternal self-compatible and paternal self-incompatible plant (SC × SI; top right), a maternal self-incompatible and paternal self-compatible plant (SI × SC; bottom left), and two self-incompatible plants (SI × SI, bottom right). Seeds are from crosses within and between the self-incompatible Greek and the self-compatible Swedish populations and illustrate the effects of cross type and cross direction on seed morphology. The scale bar is 5 mm.

t-tests to determine whether, for each trait and total reproductive isolation, crosses within (SC × SC, SI × SI) and between (SC × SI, SI × SC) breeding systems displayed significant reproductive isolation. Additionally, we tested for differences in seed and germination traits between cross directions for each of the 15 pairs of population crosses. For the number of seeds and seed mass, we constructed linear mixed models with cross direction as a fixed factor and maternal individual as a random factor. For germination, we constructed generalized linear models with cross direction as a factor and a quasibinomial error distribution (due to overdispersion) and logit as a link function. Using t-tests, we also tested for differences between cross directions in total reproductive isolation quantified as the number of viable seeds (the product of the number of seeds per fruit and the proportion of seeds germinating). For germination and the number of viable seeds, a random factor was not needed since there was only one data point for each maternal individual. For each trait, resulting *p*-values were corrected using a false discovery rate (FDR) of 5% (Benjamini & Hochberg, 1995).

Fourth, to examine the effect of seed mass on seed germination, we constructed generalized linear mixed models with proportion of seeds germinating as response variable, cross type, seed mass and their interaction as fixed factors, and population pair nested within cross type as random factor, with a beta-binomial error distribution and a logit link function. This was done in two separate models, one including only crosses within breeding systems (SC × SC, SI × SI) and one including only crosses between breeding systems (SC × SI, SI × SC). By doing so, we tested whether the effect of seed mass on germination differed between SC × SC and SI × SI crosses, and likewise whether the effect of seed mass on germination differed between the direction of the cross (SC × SI vs. SI × SC) in crosses between breeding systems.

Finally, we examined variation in plant size in the F1 generation resulting from the crosses. We tested for differences in rosette size 30 days after pots with seeds in the germination experiment were moved from stratification to the greenhouse, and after 13 weeks of vernalization, using mean values in the 87% of cases when there was both a transplanted and a non-transplanted individual from the same maternal individual available (results were similar using only non-transplanted plants). Variation in rosette size was analyzed in the same way as seed number and seed mass, with linear mixed models with cross type and cross direction as fixed factors and population pair as a random factor.

Results

Number of seeds per fruit

Cross type and the interaction between cross type and cross direction affected the number of seeds per fruit (cross type: $F_{2,11} = 12.60$, $p = .002$; cross direction: $F_{1,164} = 1.43$, $p = .23$; and interaction: $F_{2,164} = 13.94$, $p < .001$; Figure 1A). SC × SC crosses produced the highest number of seeds per fruit and SI × SI crosses produced the fewest seeds per fruit. For crosses between populations of different breeding systems (SC × SI and SI × SC), the number of seeds per fruit was affected by the direction of the cross. SC × SI crosses produced more seeds per fruit than did SI × SC crosses (Figure 1A), which is likely due to self-compatible populations producing more ovules per fruit than self-incompatible populations (*cf.* Stephenson, 1981).

RI_{Seeds} was low for crosses both between and within breeding systems, and neither the cross type, the cross direction, or their interaction had a significant effect on RI_{Seeds} (Figure 2A, Table 2, Table 3). After FDR correction, the number of seeds produced differed significantly between cross directions in five of 15 reciprocal crosses between populations, with more seeds produced when the paternal plant had a stronger genome in four of those cases (Supplementary Figure S3a, Supplementary Table S1).

Seed size

Cross type, cross direction, and their interaction affected seed mass (cross type: $F_{2,12} = 162.51$, $p < .001$; cross direction: $F_{1,158} = 146.89$, $p < .001$; and interaction: $F_{2,158} = 9.98$, $p < .001$; Figure 1B, D). Notably, crosses between breeding systems resulted in seeds that were on average 74% smaller than seeds from SC × SC and SI × SI crosses. Seed mass further differed between cross directions for all cross types, with smaller seeds produced when the maternal genome was stronger. Seed area showed a similar pattern as seed mass (Supplementary Figure S2). In 14 of the 15 reciprocal crosses between populations, seeds were larger (heavier) when the paternal plant had a stronger genome, with a statistically significant difference after FDR correction between cross directions in eight of those cases (three between and five within breeding systems; Supplementary Figure S3b and c, Supplementary Table S1).

There was a significant negative correlation between the difference in parental conflict and seed size asymmetry for crosses between breeding systems ($r = -0.90$, $p = .001$, $n = 9$; Figure 3, Supplementary Table S2), and a negative but non-significant correlation for crosses within breeding systems ($r = -0.70$, $p = 0.12$, $n = 6$; Figure 3). Because, with one exception, all asymmetry values were negative, the negative correlations indicate that seed size asymmetry increased with increasing difference in parental conflict, with the seeds from crosses with a stronger paternal than maternal genome being larger than the opposite crosses.

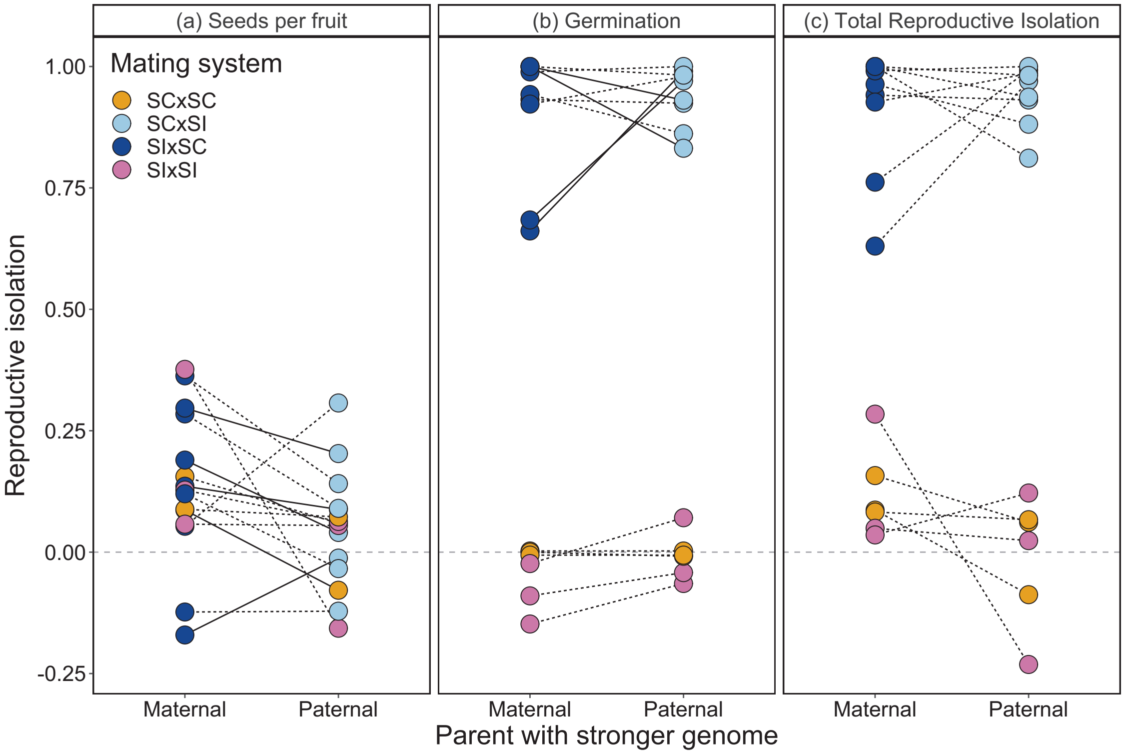

Figure 2. Estimates of reproductive isolation in terms of reductions in the number of seeds per fruit (A), proportion of seeds germinating (B), and total reproductive isolation (C) in reciprocal crosses between self-compatible populations (SC × SC, orange), between self-compatible and self-incompatible populations (SC × SI, light blue; SI × SC, dark blue), and between self-incompatible populations (SI × SI; pink) of *Arabis alpina*. A value of 0 indicates no reproductive isolation, whereas a value of 1 indicates complete reproductive isolation. Lines connect data points representing crosses between the same two populations in different directions (maternal or paternal genome predicted to be strongest; see *Methods* for details). Solid lines indicate statistically significant effects of cross direction (after false discovery rate (FDR) correction, Supplementary Table S1).

Table 2. Reproductive isolation (*RI*) based on the number of seeds per fruit, proportion of seeds germinating, and total reproductive isolation, for crosses among self-compatible (SC) populations or among self-incompatible (SI) populations (SC × SC and SI × SI), and between SC and SI populations (SC × SI and SI × SC) of *Arabis alpina*. The statistical significance of *RI* was examined with one-sample t-tests, to test if *RI* was different from zero ($p < .05$ indicated in bold).

	Seeds per fruit				Germination				Total *RI*			
	RI	*t*	*df*	*p*	*RI*	*t*	*df*	*p*	*RI*	*t*	*df*	*p*
SC × SC, SI × SI	0.08	4.60	5	**0.0058**	−0.03	−1.30	5	0.25	0.054	3.29	5	**0.022**
SC × SI, SI × SC	0.10	2.26	8	0.054	0.92	44.14	8	**<0.001**	0.93	45.10	8	**<0.001**

Table 3. Effects of cross type (between populations of the same breeding system, SC × SC or SI × SI vs. between populations with different breeding system, SC × SI or SI × SC) and cross direction (maternal vs. paternal plant having a stronger genome) on reproductive isolation (*RI*) calculated based on the number of seeds per fruit and proportion of seeds germinating, and total reproductive isolation for *Arabis alpina*. Effects were examined with two-way ANOVAs. Significant effects ($p < .05$) are indicated in bold.

	Seeds per fruit *RI*			Germination *RI*			Total *RI*		
	df	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
Cross Type (CT)	1,26	0.27	0.61	1,26	830.2	**<0.001**	1,26	486.7	**<0.001**
Cross Direction (CD)	1,26	2.93	0.099	1,26	1.3	0.26	1,26	0.64	0.43
CT × CD	1,26	0.84	0.37	1,26	0.001	0.98	1,26	3.72	0.063

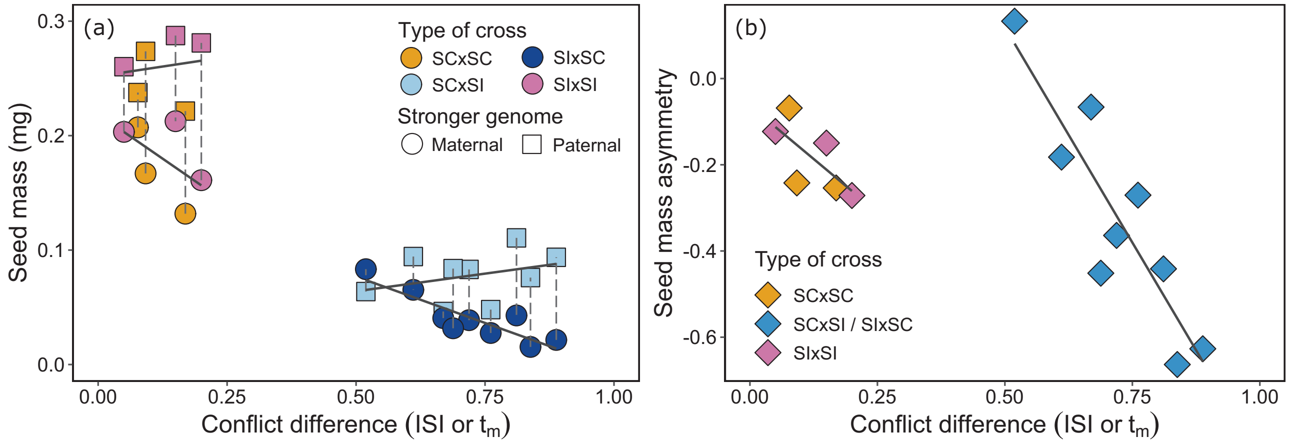

Figure 3. Relationship between the magnitude of parental conflict difference, quantified as the difference in index of self-incompatibility (ISI) or outcrossing rate (t_m) and (A) mean seed mass after reciprocal crosses between pairs of *Arabis alpina* populations, and (B) seed mass asymmetry in these reciprocal crosses. The symbol color indicates the type of cross. In (A), reciprocal crosses are connected with a dashed line, with symbol shape indicating the direction of the cross (maternal or paternal population having a stronger genome). In (B), a negative seed mass asymmetry indicates that seeds were larger in the cross direction with the paternal parent having a stronger genome.

Seed germination

Germination was affected by cross type but not cross direction or their interaction (cross type: $\chi^2_2 = 164.92$, $p < .001$; cross direction: $\chi^2_1 = 0.69$, $p = .41$; and interaction: $\chi^2_2 = 3.78$, $p = .15$; Figure 1C). While germination was high for crosses within breeding systems (SC × SC: 98%; SI × SI: 83%), only 7% of seeds from crosses between breeding systems (SC × SI and SI × SC) germinated.

Only the type of cross had a significant effect on $RI_{Germination}$, with higher and statistically significant RI for crosses between breeding systems (Figure 2B, Table 2, and Table 3). After FDR correction, germination differed significantly between cross directions in four of 15 reciprocal crosses between populations (all between breeding systems), with higher germination when the maternal plant had a stronger genome in two of those cases (Supplementary Figure S3d, Supplementary Table S1).

No significant effect of seed mass on germination was detected for crosses between populations of the same breeding system (SC × SC and SI × SI; Table 4). In contrast, for crosses between breeding systems (SC × SI and SI × SC), germination increased with seed mass (beyond a minimum seed mass associated with any germination at all), and did so more rapidly for SI × SC crosses compared to SC × SI crosses (statistically significant seed mass × cross-type interaction; Figure 4, Table 4).

Plant size

Cross type, cross direction or their interaction had no statistically significant effect on leaf rosette size after 30 days of growth (cross type: $F_{2,5} = 5.13$, $P = 0.061$; cross direction: $F_{1,94} = 0.36$, $p = .55$; and interaction: $F_{2,92} = 0.37$, $p = .69$; Supplementary Figure S4a), or after the vernalization treatment (cross type: $F_{2,5}$ = 1.67, p = .28; cross direction: $F_{1,98}$ = 0.093, p = .76; and interaction: $F_{2,97}$ = 1.03, p = .36; Supplementary Figure S4b).

Total reproductive isolation

Our estimate of total post-pollination reproductive isolation (based on a combination of the number of seeds and proportion of seeds germinating) was high after crosses between populations of different breeding systems, and low after crosses between populations of the same breeding system (Figure 2C, Table 2). Consequently, only the type of cross, but not the direction of the cross or their interaction, had a significant effect on RI_{Total} (Table 3). In the 15 reciprocal crosses, there was no significant effect of cross direction on the number of viable seeds per fruit after FDR correction (Supplementary Table S1).

Table 4. Effects of seed mass and cross type on the proportion of seeds germinating in crosses within and between breeding systems of *Arabis alpina*, examined with generalized linear mixed models. For within-breeding system crosses (random effect *SD* = 0.00028), cross type contrasts crosses among self-compatible (SC) vs. among self-incompatible (SI) populations (SC × SC vs. SI × SI). For between-breeding system crosses (random effect *SD* = 0.0023), cross type denotes the direction of cross (SC × SI vs. SI × SC). Significant effects (p < .05) are indicated in bold.

	Within breeding system			Between breeding system		
Fixed effects	*df*	χ2	*p*	*df*	χ2	*p*
Seed mass (SM)	1	0.39	0.53	1	59.5	**<0.001**
Cross type (CT)	1	10.9	**0.001**	1	27.6	**<0.001**
SM × CT	1	1.30	0.25	1	15.5	**<0.001**

Discussion

In this study, we have documented strong reproductive isolation between self-compatible and self-incompatible populations of the perennial herb *A. alpina*. Crosses between populations of different breeding systems resulted in markedly smaller seeds with lower germination success compared to crosses between populations of the same breeding system. Moreover, in 14 of 15 reciprocal crosses, seeds were heavier when the paternal plant was predicted to have a stronger genome than the maternal plant, and the magnitude in seed size asymmetry between cross directions increased with the magnitude of the predicted difference in parental conflict. These results are consistent with higher levels of parental conflict in more outcrossing populations and suggest that such conflicts may contribute to the evolution of reproductive isolation between plant populations of different mating/breeding systems.

Parental conflict differences and seed development in crosses within breeding systems

Crosses between populations with the same breeding system were associated with comparatively small differences in predicted parental conflict (genome strength) and weak reproductive isolation, but cross direction still had a significant effect on seed size. Consistent with the WISO hypothesis (Brandvain & Haig, 2005), crosses where the maternal genome was stronger resulted in somewhat smaller seeds, while crosses with a stronger paternal genome resulted in somewhat larger seeds (Figure 1). Similar directional differences in seed size have previously been found when crossing populations with different mating systems in *Dalechampia scandens* (Raunsgard et al., 2018) and *A. lyrata* (Willi, 2013). Importantly, *A. alpina* seeds produced after crosses between populations of the same breeding system were not, on average for both cross directions, smaller than seeds that were

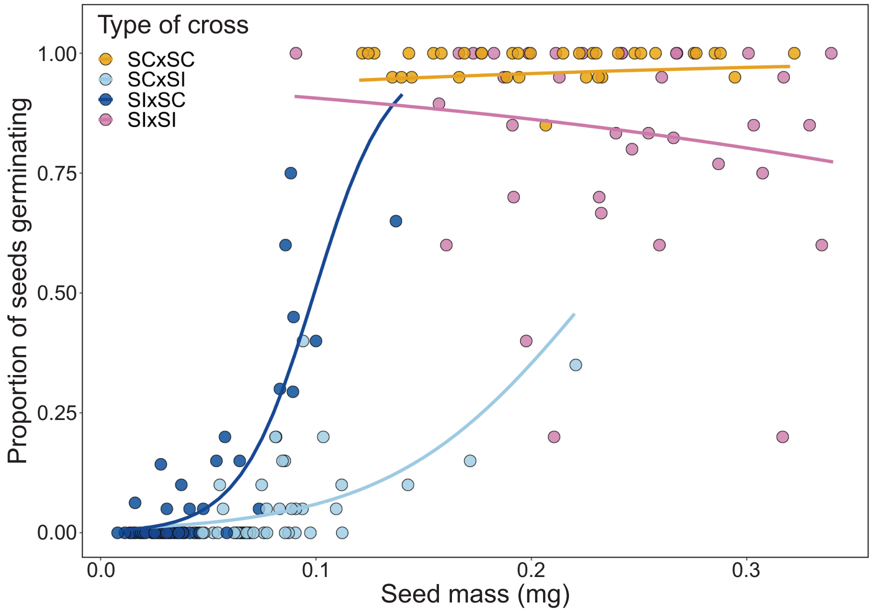

Figure 4. The proportion of seeds germinating as a function of seed mass and type of cross in *Arabis alpina*. Crosses were made between self-compatible populations (SC × SC, orange), between self-incompatible populations (SI × SI; pink), and between self-compatible and self-incompatible populations (SC × SI, light blue; SI × SC, dark blue). Data points show the proportion of seeds germinating for individual maternal plants, lines indicate model predictions for each cross type from the generalized linear mixed models in Table 4.

the product of crosses within populations (Supplementary Figure S3b and c). Additionally, these seeds had high levels of germination regardless of seed size (Figure 4, Table 4), and our estimates of total reproductive isolation were low (mean RI_{Total} = 0.054). This indicates that the limited differences in the parental conflict in these crosses generate little to no reproductive isolation.

Parental conflict differences and seed development in crosses between breeding systems

Differences in the predicted parental conflict were larger in crosses between breeding systems, and these crosses indicated strong reproductive isolation due to reduced seed size and germination success. In line with previous empirical studies in *Capsella* (Lafon-Placette et al., 2018; Rebernig et al., 2015), *Arabidopsis* (Lafon-Placette et al., 2017), *Solanum* (Roth et al., 2018), and *Mimulus* (Coughlan et al., 2020), seeds resulting from crosses between self-compatible and self-incompatible populations were larger when the paternal genome was stronger than when the cross was conducted in the opposite direction. Moreover, the magnitude of the seed size asymmetry increased with increasing difference in parental conflict (blue diamonds in Figure 3B). In both cross directions, seeds were substantially smaller and the proportion of seeds germinating was greatly reduced compared to crosses within breeding systems (Figure 1). Our estimates of total reproductive isolation were very high for crosses between self-compatible and self-incompatible populations (mean RI_{Total} = 0.93), and this was mainly a function of effects manifested at the germination stage (Figure 2, Table 2). As a comparison, interspecific crosses in other Brassicaceae genera (*Leavenworthia*, Koelling & Mauricio, 2010; *Cakile* and *Erucaria*, Willis & Donohue, 2017) showed less post-pollination reproductive isolation than crosses between breeding systems in *A. alpina*. Hence, using the biological species concept, self-compatible and self-incompatible *A. alpina* populations in Europe are reproductively isolated to an extent that they may be considered different species.

Relationship between difference in parental conflict and seed size is non-linear when the paternal genome is stronger

Our findings are consistent with the theoretical prediction that crosses involving a substantially stronger maternal genome (SI × SC) should yield smaller seeds owing to the endosperm receiving limited resources due to precocious cellularization, and that endosperm cellularization may fail after crosses with a substantially stronger paternal genome (SC × SI), resulting in early abortion and a deformed but slightly larger seed (Brandvain & Haig, 2005; Coughlan et al., 2020; Gehring & Satyaki, 2017; Haig & Westoby, 1991; Lafon-Placette & Köhler, 2016; Städler et al., 2021). Seeds produced in crosses where the maternal genome was strongest showed a steady decrease in size with increasing conflict difference (considering crosses both within and between breeding systems; Figure 3A). In contrast, seeds resulting from crosses with a stronger paternal genome showed a drastic drop in size when crosses within and between breeding systems are compared, and little variation among crosses within breeding systems. These results suggest that in *A. alpina*, endosperm cellularization may fail due to a stronger paternal genome at a threshold conflict difference of 0.25–0.5, which causes the "reset" in seed size asymmetry observed in Figure 3B. More generally, our results suggest that the relationship between the difference in parental conflict and seed size is not necessarily linear.

The relationship between seed size and germination in crosses between breeding systems

The effect of seed size on germination differed between cross directions in crosses between breeding systems. Although germination was overall very low, it increased more rapidly with seed size for SI × SC crosses compared to SC × SI crosses (compare dark and light blue lines in Figure 4). This suggests that, for crosses with a stronger maternal genome, most seeds are simply too small to be viable. At slightly higher seed masses (around 0.1 mg), germination success increased rapidly, indicative of a functioning endosperm. In contrast, with a stronger paternal genome, seeds were mostly inviable even at sizes above 0.1 mg, suggesting a failed endosperm development (Lafon-Placette & Köhler, 2016). Hence, parental conflict differences may reduce seed germination via different mechanisms depending on cross direction, resulting in low germination in both cases. In other studies of outcrossing and selfing plants, seed viability/germination was reduced with a stronger maternal genome (Ruhsam et al., 2011; Willis & Donohue, 2017), with a stronger paternal genome (Rebernig et al., 2015), or in both directions (Fishman & Stratton, 2004; Oneal et al., 2016). Our results demonstrate that in crosses between plants with different levels of predicted parental conflict, the magnitude of the conflict difference may be the key factor influencing seed development and germination success.

Other factors potentially causing reproductive isolation

It can be difficult to distinguish the effects of parental conflict from other factors affecting reproductive isolation (*cf.* Bushell et al., 2003). Bateson–Dobzhansky–Muller incompatibilities between nuclear genes without parent-of-origin effects (Rieseberg & Blackman, 2010; Sweigart & Willis, 2012) or in the form of cytonuclear interactions (Caruso et al., 2012; Roux et al., 2016; Tiffin et al., 2001), could potentially contribute to the strong reproductive isolation between self-compatible and self-incompatible *A. alpina* populations. However, the effect of cross direction on seed size in crosses between populations of different breeding systems is consistent with an asymmetric effect of parental conflict rather than symmetric incompatibilities (Städler et al., 2021). Additionally, Hedberg (1962, p. 254) noted that reciprocal crosses between Swedish and Kenyan populations of *A. alpina*, both of which are self-compatible and selfing, "*gave good seed setting in both directions, and the seeds germinated very well*," despite these populations belonging to separate lineages (Ehrich et al., 2007; Koch et al., 2006; Wötzel et al., 2021). This suggests that parental conflict differences, rather than other genetic differentiation (due to divergent selection or genetic drift), may explain incompatibilities between breeding systems in *A. alpina*. Studies of endosperm development and gene expression after controlled crosses could be used to investigate this further (İltaş et al., 2021; Lafon-Placette et al., 2017; Rebernig et al., 2015).

Conclusions

To conclude, our results suggest that parental conflict over seed provisioning varies consistently between populations of different mating systems in *A. alpina*. As a result, while crosses between populations within breeding systems produce viable seeds, crosses between breeding systems, which are predicted to have larger differences in parental conflict, result in small, largely inviable seeds and strong reproductive isolation to an extent that self-compatible and self-incompatible *A. alpina* populations in Europe may be considered different species. Our study adds to previous results (e.g., Coughlan et al., 2020; Lafon-Placette et al., 2017; Ruhsam et al., 2011) by suggesting that differences in parental conflict can, depending on their magnitude, result in strong post-pollination reproductive barriers in flowering plants, with potential consequences for hybridization, gene flow, and ultimately speciation.

Supplementary material

Supplementary material is available online at *Evolution* (https://academic.oup.com/evolut/qpad016)

Data availability

Data and scripts are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.7d7wm3808 (Petrén et al., 2023).

Author contributions

H.P., M.F., P.T., and J.Å. planned and designed the research; H.P., M.F., and H.T. collected data; H.P. performed statistical analyses with advice from J.Å, M.F., and M.S.; H.P. wrote the manuscript with input from M.F., J.Å., M.S., and contributions from all authors.

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

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References

Ansell, S. W., Grundmann, M., Russell, S. J., Schneider, H., & Vogel, J. C. (2008). Genetic discontinuity, breeding-system change and population history of *Arabis alpina* in the Italian Peninsula and adjacent Alps. *Molecular Ecology*, 17(9), 2245–2257. https://doi.org/10.1111/j.1365-294X.2008.03739.x

Barrett, S. C. H. (2002). The evolution of plant sexual diversity. *Nature Reviews Genetics*, 3(4), 274–284. https://doi.org/10.1038/nrg776

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48. https://doi.org/10.18637/jss.v067.i01

Bateson, W. (1909). Heredity and variation in modern lights. In A. C. Seward (Ed.), *Darwin and modern science: Essays in commemoration of the centenary of the birth of Charles Darwin and of the fiftieth anniversary of the publication of the Origin of Species* (pp. 85–101). Cambridge University Press.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B Methodology*, 57, 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Brandvain, Y., & Haig, D. (2005). Divergent mating systems and parental conflict as a barrier to hybridization in flowering plants. *American Naturalist*, 166(3), 330–338. https://doi.org/10.1086/432036

Briscoe Runquist, R. D., Chu, E., Iverson, J. L., Kopp, J. C., & Moeller, D. A. (2014). Rapid evolution of reproductive isolation between incipient outcrossing and selfing *Clarkia* species. *Evolution*, 68(10), 2885–2900. https://doi.org/10.1111/evo.12488

Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Machler, M., & Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, 9, 378–400. https://doi.org/10.32614/RJ-2017-066

Buehler, D., Graf, R., Holderegger, R., & Gugerli, F. (2012). Contemporary gene flow and mating system of *Arabis alpina* in a Central European alpine landscape. *Annals of Botany*, 109(7), 1359–1367. https://doi.org/10.1093/aob/mcs066

Burt, A., & Trivers, R. (2006). *Genes in conflict: The biology of selfish genetic elements*. Belknap Press of Harvard University Press.

Bushell, C., Spielman, M., & Scott, R. J. (2003). The basis of natural and artificial postzygotic hybridization barriers in *Arabidopsis* species. *Plant Cell*, 15(6), 1430–1442. https://doi.org/10.1105/tpc.010496

Caruso, C. M., Case, A. L., & Bailey, M. F. (2012). The evolutionary ecology of cytonuclear interactions in angiosperms. *Trends in Plant Science*, 17(11), 638–643. https://doi.org/10.1016/j.tplants.2012.06.006

Coughlan, J. M., Wilson Brown, M., & Willis, J. H. 2020. Patterns of hybrid seed inviability in the *Mimulus guttatus* sp. complex reveal a potential role of parental conflict in reproductive isolation. *Current Biology*, 30(1), 83–93.e5. https://doi.org/10.1016/j.cub.2019.11.023

Coyne, J. A., & Orr, H. A. (1989). Patterns of speciation in *Drosophila*. *Evolution*, 43(2), 362–381. https://doi.org/10.1111/j.1558-5646.1989.tb04233.x

Cutter, A. D. (2019). Reproductive transitions in plants and animals: Selfing syndrome, sexual selection and speciation. *New Phytologist*, 224(3), 1080–1094. https://doi.org/10.1111/nph.16075

Dobzhansky, T. (1936). Studies on hybrid sterility. II. Localization of sterility factors in *Drosophila pseudoobscura* hybrids. *Genetics*, 21(2), 113–135. https://doi.org/10.1093/genetics/21.2.113

Egido, L. L., Toorop, P. E., & Lanfermeijer, F. C. (2018). Seed priming improves germination of *Arabis alpina* under thermo-inhibiting conditions. *Seed Science and Technology*, 46, 285–303. https://doi.org/10.15258/sst.2018.46.2.10

Ehrich, D., Gaudeul, M., Assefa, A., Koch, M. A., Mummenhoff, K., Nemomissa, S., Consortium, I., & Brochmann, C. (2007). Genetic consequences of Pleistocene range shifts: Contrast between the Arctic, the Alps and the East African mountains. *Molecular Ecology*, 16(12), 2542–2559. https://doi.org/10.1111/j.1365-294x.2007.03299.x

Fishman, L., & Stratton, D. A. (2004). The genetics of floral divergence and postzygotic barriers between outcrossing and selfing populations of *Arenaria uniflora* (Caryophyllaceae). *Evolution*, 58(2), 296–307. https://doi.org/10.1111/j.0014-3820.2004.tb01646.x

Foxe, J. P., Slotte, T., Stahl, E. A., Neuffer, B., Hurka, H., & Wright, S. I. (2009). Recent speciation associated with the evolution of selfing in *Capsella*. *Proceedings of the National Academy of Sciences*, 106, 5241–5245. https://doi.org/10.1073/pnas.0807679106

Garner, A. G., Kenney, A. M., Fishman, L., & Sweigart, A. L. (2016). Genetic loci with parent-of-origin effects cause hybrid seed lethality in crosses between *Mimulus* species. *New Phytologist*, 211(1), 319–331. https://doi.org/10.1111/nph.13897

Gehring, M., & Satyaki, P. R. (2017). Endosperm and imprinting, inextricably linked. *Plant Physiology*, 173(1), 143–154. https://doi.org/10.1104/pp.16.01353

Geist, K. S., Strassmann, J. E., & Queller, D. C. (2019). Family quarrels in seeds and rapid adaptive evolution in *Arabidopsis*. *Proceedings of the National Academy of Sciences*, 116, 9463–9468. https://doi.org/10.1073/pnas.181773311

Gorman, C. E., Li, Y., Dorken, M. E., & Stift, M. (2021). No evidence for incipient speciation by selfing in North American *Arabidopsis lyrata*. *Journal of Evolutionary Biology*, 34(9), 1397–1405. https://doi.org/10.1111/jeb.13901

Haig, D., & Westoby, M. (1989). Parent-specific gene expression and the triploid endosperm. *American Naturalist*, 134(1), 147–155. https://doi.org/10.1086/284971

Haig, D., & Westoby, M. (1991). Genomic imprinting in endosperm: Its effect on seed development in crosses between species, and between different ploidies of the same species, and its implications for the evolution of apomixis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 333, 1–13. https://doi.org/10.1098/rstb.1991.0057

Harrison, X. A. (2015). A comparison of observation-level random effect and Beta-Binomial models for modelling overdispersion in Binomial data in ecology & evolution. *PeerJ*, 3, e1114. https://doi.org/10.7717/peerj.1114

Hedberg, O. (1962). Intercontinental crosses in *Arabis Alpina* L. *Caryologia*, 15(2), 253–260. https://doi.org/10.1080/00087114.1962.10796058

İltaş, Ö., Svitok, M., Cornille, A., Schmickl, R., & Lafon Placette, C. (2021). Early evolution of reproductive isolation: A case of weak inbreeder/strong outbreeder leads to an intraspecific hybridization barrier in *Arabidopsis lyrata*. *Evolution*, 75, 1466–1476. https://doi.org/10.1111/evo.14240

Johnston, S. A., Den Nijs, T. P. M., Peloquin, S. J., & Hanneman, R. E. (1980). The significance of genic balance to endosperm development in interspecific crosses. *Theoretical and Applied Genetics*, 57(1), 5–9. https://doi.org/10.1007/bf00276002

de Jong, T. J., & Scott, R. J. (2007). Parental conflict does not necessarily lead to the evolution of imprinting. *Trends in Plant Science*, 12(10), 439–443. https://doi.org/10.1016/j.tplants.2007.07.003

Koch, M. A., Kiefer, C., Ehrich, D., Vogel, J., Brochmann, C., & Mummenhoff, K. (2006). Three times out of Asia Minor: The phylogeography of *Arabis alpina* L. (Brassicaceae). *Molecular Ecology*, 15(3), 825–839. https://doi.org/10.1111/j.1365-294X.2005.02848.x

Koelling, V. A., & Mauricio, R. (2010). Genetic factors associated with mating system cause a partial reproductive barrier between two parapatric species of *Leavenworthia* (Brassicaceae). *American Journal of Botany*, 97(3), 412–422. https://doi.org/10.3732/ajb.0900184

Köhler, C., Dziasek, K., & Del Toro-De León, G. (2021). Postzygotic reproductive isolation established in the endosperm: Mechanisms, drivers and relevance. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 376(1826), 20200118. https://doi.org/10.1098/rstb.2020.0118.

Kondoh, M., & Higashi, M. (2000). Reproductive isolation mechanism resulting from resolution of intragenomic conflict. *American Naturalist*, 156(5), 511–518. https://doi.org/10.1086/303409

Krannitz, P. G., Aarssen, L. W., & Dow, J. M. (1991). The effect of genetically based differences in seed size on seedling survival in *Arabidopsis thaliana* (Brassicaceae). *American Journal of Botany*, 78(3), 446–450. https://doi.org/10.1002/j.1537-2197.1991.tb15207.x

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, 82, 1–26. https://doi.org/10.18637/jss.v082.i13

Laenen, B., Tedder, A., Nowak, M. D., Toräng, P., Wunder, J., Wötzel, S., Steige, K. A., Kourmpetis, Y., Odong, T., Drouzas, A. D., Bink, M. C. A. M., Ågren, J., Coupland, G., & Slotte, T. (2018). Demography and mating system shape the genome-wide impact of purifying selection in *Arabis alpina*. *Proceedings of the National Academy of Sciences*, 115, 816–821. https://doi.org/10.1073/pnas.1707492115

Lafon-Placette, C., Hatorangan, M. R., Steige, K. A., Cornille, A., Lascoux, M., Slotte, T., & Köhler, C. (2018). Paternally expressed imprinted genes associate with hybridization barriers in *Capsella*. *Nature Plants*, 4(6), 352–357. https://doi.org/10.1038/s41477-018-0161-6

Lafon-Placette, C., Johannessen, I. M., Hornslien, K. S., Ali, M. F., Bjerkan, K. N., Bramsiepe, J., Glöckle, B. M., Rebernig, C. A., Brysting, A. K., Grini, P. E., & Köhler, C. (2017). Endosperm-based hybridization barriers explain the pattern of gene flow between *Arabidopsis lyrata* and *Arabidopsis arenosa* in Central Europe. *Proceedings of the National Academy of Sciences*, 114, E1027–E1035. https://doi.org/10.1073/pnas.1615123114

Lafon-Placette, C., & Köhler, C. (2016). Endosperm-based postzygotic hybridization barriers: Developmental mechanisms and evolutionary drivers. *Molecular Ecology*, 25(11), 2620–2629. https://doi.org/10.1111/mec.13552

Lenth, R. (2022). *emmeans: Estimated Marginal Means, aka least-squares means*. R package version 1.8.0. https://CRAN.R-project.org/package=emmeans

Lewis, D., & Crowe, L. K. (1958). Unilateral interspecific incompatibility in flowering plants. *Heredity*, 12(2), 233–256. https://doi.org/10.1038/hdy.1958.26

Li, J., & Berger, F. (2012). Endosperm: Food for humankind and fodder for scientific discoveries. *New Phytologist*, 195(2), 290–305. https://doi.org/10.1111/j.1469-8137.2012.04182.x

Lloyd, D. G. (1968). Partial unilateral incompatibility in *Leavenworthia* (Cruciferae). *Evolution*, 22(2), 382–393. https://doi.org/10.1111/j.1558-5646.1968.tb05905.x

Lowry, D. B., Modliszewski, J. L., Wright, K. M., Wu, C. A., & Willis, J. H. (2008). The strength and genetic basis of reproductive isolating barriers in flowering plants. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363, 3009–3021. https://doi.org/10.1098/rstb.2008.0064

Martin, N. H., & Willis, J. H. (2007). Ecological divergence associated with mating system causes nearly complete reproductive isolation between sympatric *Mimulus* species. *Evolution*, 61(1), 68–82. https://doi.org/10.1111/j.1558-5646.2007.00006.x

Muller, H. J. (1942). Isolating mechanisms, evolution and temperature. *Biology Symposium*, 6, 71–125.

Oneal, E., Willis, J. H., & Franks, R. G. (2016). Disruption of endosperm development is a major cause of hybrid seed inviability between *Mimulus guttatus* and *Mimulus nudatus*. *New Phytologist*, 210(3), 1107–1120. https://doi.org/10.1111/nph.13842

Petrén, H., Thosteman, H., Stift, M., Toräng, P., Ågren, J., & Friberg, M. (2023). *Differences in mating system and predicted parental conflict affect post-pollination reproductive isolation in a flowering plant*. Dryad, Dataset. https://doi.org/10.5061/dryad.7d7wm3808

Petrén, H., Toräng, P., Ågren, J., & Friberg, M. (2021). Evolution of floral scent in relation to self-incompatibility and capacity for autonomous self-pollination in the perennial herb *Arabis alpina*. *Annals of Botany*, 127(6), 737–747. https://doi.org/10.1093/aob/mcab007

Pickup, M., Brandvain, Y., Fraïsse, C., Yakimowski, S., Barton, N. H., Dixit, T., Lexer, C., Cereghetti, E., & Field, D. L. (2019). Mating system variation in hybrid zones: Facilitation, barriers and asymmetries to gene flow. *New Phytologist*, 224(3), 1035–1047. https://doi.org/10.1111/nph.16180

Queller, D. C. (1983). Kin selection and conflict in seed maturation. *Journal of Theoretical Biology*, 100(1), 153–172. https://doi.org/10.1016/0022-5193(83)90099-1

R Core Team. (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Raduski, A. R., Haney, E. B., & Igić, B. (2012). The expression of self-incompatibility in angiosperms is bimodal. *Evolution*, 66(4), 1275–1283. https://doi.org/10.1111/j.1558-5646.2011.01505.x

Raunsgard, A., Opedal, H., Ekrem, R. K., Wright, J., Bolstad, G. H., Armbruster, W. S., & Pélabon, C. (2018). Intersexual conflict over seed size is stronger in more outcrossed populations of a mixed-mating plant. *Proceedings of the National Academy of Sciences*, 115, 11561–11566. https://doi.org/10.1073/pnas.1810979115

Rebernig, C. A., Lafon-Placette, C., Hatorangan, M. R., Slotte, T., & Köhler, C. (2015). Non-reciprocal interspecies hybridization barriers in the *Capsella* genus are established in the endosperm. *PLoS Genetics*, 11(6), e1005295. https://doi.org/10.1371/journal.pgen.1005295

Rieseberg, L. H., & Blackman, B. K. (2010). Speciation genes in plants. *Annals of Botany*, 106(3), 439–455. https://doi.org/10.1093/aob/mcq126

Roth, M., Florez-Rueda, A. M., Griesser, S., Paris, M., & Städler, T. (2018). Incidence and developmental timing of endosperm failure in post-zygotic isolation between wild tomato lineages. *Annals of Botany*, 121(1), 107–118. https://doi.org/10.1093/aob/mcx133

Roux, F., Mary-Huard, T., Barillot, E., Wenes, E., Botran, L., Durand, S., Villoutreix, R., Martin-Magniette, M. -L., Camilleri, C., & Budar, F. (2016). Cytonuclear interactions affect adaptive traits of the annual plant *Arabidopsis thaliana* in the field. *Proceedings of the National Academy of Sciences*, 113, 3687–3692. https://doi.org/10.1073/pnas.1520687113

Ruhsam, M., Hollingsworth, P. M., & Ennos, R. A. (2011). Early evolution in a hybrid swarm between outcrossing and selfing lineages in *Geum*. *Heredity*, 107(3), 246–255. https://doi.org/10.1038/hdy.2011.9

Sandstedt, G. D., Wu, C. A., & Sweigart, A. L. (2021). Evolution of multiple postzygotic barriers between species of the *Mimulus tilingii* complex. *Evolution*, 75(3), 600–613. https://doi.org/10.1111/evo.14105

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9(7), 671–675. https://doi.org/10.1038/nmeth.2089

Sicard, A., & Lenhard, M. (2011). The selfing syndrome: A model for studying the genetic and evolutionary basis of morphological adaptation in plants. *Annals of Botany*, 107(9), 1433–1443. https://doi.org/10.1093/aob/mcr023

Smith, C. C., & Fretwell, S. D. (1974). The optimal balance between size and number of offspring. *American Naturalist*, 108(962), 499–506. https://doi.org/10.1086/282929

Städler, T., Florez-Rueda, A. M., & Roth, M. (2021). A revival of effective ploidy: The asymmetry of parental roles in endosperm-based hybridization barriers. *Current Opinion in Plant Biology*, 61, 102015. https://doi.org/10.1016/j.pbi.2021.102015

Stebbins, G. L. (1957). Self fertilization and population variability in the higher plants. *American Naturalist*, 91(861), 337–354. https://doi.org/10.1086/281999

Stephenson, A. G. (1981). Flower and fruit abortion: Proximate causes and ultimate functions. *Annual Review of Ecology and Systematics*, 12(1), 253–279. https://doi.org/10.1146/annurev.es.12.110181.001345

Susko, D. J., & Lovett-Doust, L. (2000). Patterns of seed mass variation and their effects on seedling traits in *Alliaria petiolata* (Brassicaceae). *American Journal of Botany*, 87(1), 56–66. https://doi.org/10.2307/2656685

Sweigart, A. L., & Willis, J. H. (2012). Molecular evolution and genetics of postzygotic reproductive isolation in plants. *F1000 Biology Reports*, 4, 23. https://doi.org/10.3410/B4-23

Tedder, A., Ansell, S. W., Lao, X., Vogel, J. C., & Mable, B. K. (2011). Sporophytic self-incompatibility genes and mating system variation in *Arabis alpina*. *Annals of Botany*, 108(4), 699–713. https://doi.org/10.1093/aob/mcr157

Tedder, A., Carleial, S., Gołlębiewska, M., Kappel, C., Shimizu, K. K., & Stift, M. (2015). Evolution of the selfing syndrome in *Arabis alpina* (Brassicaceae). *PLoS One*, 10, e0126618. https://doi.org/10.1371/journal.pone.0126618

Tiffin, P., Olson, M. S., & Moyle, L. E. (2001). Asymmetrical crossing barriers in angiosperms. *Proceedings of the Royal Society London B Biological Sciences*, 268, 861–867. https://doi.org/10.1098/rspb.2000.1578

Toräng, P., Vikström, L., Wunder, J., Wötzel, S., Coupland, G., & Ågren, J. (2017). Evolution of the selfing syndrome: Anther orientation and herkogamy together determine reproductive assurance in a self-compatible plant. *Evolution*, 71(9), 2206–2218. https://doi.org/10.1111/evo.13308

Trivers, R. L. (1974). Parent-offspring conflict. *American Zoologist*, 14(1), 249–264. https://doi.org/10.1093/icb/14.1.249

Turelli, M., & Moyle, L. C. (2007). Asymmetric postmating isolation: Darwin's corollary to Haldane's rule. *Genetics*, 176(2), 1059–1088. https://doi.org/10.1534/genetics.106.065979

Willi, Y. (2013). The battle of the sexes over seed size: Support for both kinship genomic imprinting and interlocus contest evolution. *American Naturalist*, 181(6), 787–798. https://doi.org/10.1086/670196

Willis, C. G., & Donohue, K. (2017). The evolution of intrinsic reproductive isolation in the genus *Cakile* (Brassicaceae). *Journal of Evolutionary Biology*, 30(2), 361–376. https://doi.org/10.1111/jeb.13011

Wötzel, S., Andrello, M., Albani, M. C., Koch, M. A., Coupland, G., & Gugerli, F. (2021). *Arabis alpina*: A perennial model plant for ecological genomics and life-history evolution. *Molecular Ecology Resources*, 22(2): 468–486. https://doi.org/10.1111/1755-0998.13490.

Wright, S. I., Kalisz, S., & Slotte, T. (2013). Evolutionary consequences of self-fertilization in plants. *Proceedings of the Royal Society Biological Sciences*, 280, 20130133. https://doi.org/10.1098/rspb.2013.0133