



UPPSALA
UNIVERSITET

Fitness Consequences of Intra- and Interregional Crosses in an
Alpine Herb, *Arabis alpina*



Kim Karlsson Moritz

Degree project in biology, Master of science (2 years), 2012

Examensarbete i biologi 45 hp till masterexamen, 2012

Biology Education Centre

Supervisors: Per Toräng and Jon Ågren

External opponent: Camille Madec

Table of contents

SUMMARY.....	2
INTRODUCTION	
Background.....	3
Mating barriers.....	3
Heterosis.....	3
The WISO hypothesis.....	3
The present study.....	4
MATERIALS AND METHODS	
Study system.....	4
Plant material.....	5
Seed output and germination.....	5
Vegetative stage.....	5
Analyses.....	5
RESULTS	
Seed characters.....	6
Vegetative characters.....	11
DISCUSSION	
Seed mass.....	15
The WISO hypothesis.....	15
Seed number.....	16
Germination.....	16
Vegetative growth.....	17
Conclusions.....	17
Further work.....	18
ACKNOWLEDGEMENTS.....	18
REFERENCES.....	18

Abstract

Separated populations differentiate over time and reproductive barriers may arise. Plants restricted to alpine regions pose suitable study systems for divergence because of the high degree of isolation. In this experiment, an investigation of fitness-related traits in crosses between five (two Scandinavian, one French and two Spanish) populations of *Arabis alpina* was carried out by comparing inter- and intrapopulation crosses. Seed mass and rosette size for the offspring plants were scored. In my study, offspring of Scandinavian maternal plants were of higher seed mass if pollinated by Spanish plants than if Spanish maternal plants were pollinated by Scandinavian plants. Seed mass correlated positively to early rosette diameters. Offspring of Scandinavian maternal plants also had wider early rosette diameters if pollinated by Spanish paternal plants than if the same maternal plants were pollinated by Scandinavian paternal plants. I also discuss these results in the context of paternal conflict.

Introduction

Background

Allopatric populations with low gene flow may over time differentiate through genetic drift and divergent selection. Given enough time, genetic reproductive barriers between populations may evolve. The evolution of reproductive isolation is a key component of speciation and includes both pre- and postzygotic isolation. One reproductive barrier is outbreeding depression (decreased hybrid vigour). However, offspring produced by crosses of two isolated lines may also exhibit heterosis (increased hybrid vigour). There is a substantial body of studies investigating hybrid vigour and outbreeding depression resulting from crosses between plant populations. Many studies have examined a single plant species inhabiting a wide geographical range (*e.g.* Barth *et al.* 2003, Etterson *et al.* 2007, Leinonen *et al.* 2011). Additionally, studies of hybridization between different species or subspecies have also been conducted (*e.g.* Andersson 1990, 1996).

Mating barriers

Failed crosses between plant populations can be due to either pre- or postzygotic isolation. The former is often accredited as the stronger, but not necessarily the first evolved among mating barriers (Widmer *et al.* 2008). Intrinsic postzygotic isolation may be expressed as reduced hybrid offspring fitness and may be due to cytonuclear incompatibilities (Bomblies 2010). Several extrinsic factors may contribute to reproductive isolation (Coyne and Orr 2004). Additionally, evolution of self-fertilization in plants may increase reproductive isolation (Widmer *et al.* 2008) and allow for yet more differentiation. Extensive divergence between populations could lead to reduced hybrid fitness.

Heterosis

Heterosis, or increased hybrid vigour, has been observed in the F₁ generations in several studies (*e.g.* Rohde *et al.* 2004, Barth *et al.* 2003). Birchler *et al.* (2010) define heterosis as offspring from a cross having enhanced developmental speed, biomass and/or fertility compared to both parents, whereas other studies use mid-parent heterosis, where offspring traits are compared to the parent mean value (*e.g.* Lamb *et al.* 1993). The term has also been used in a wider sense and was for example applied when Rohde *et al.* (2004) discovered increased frost tolerance in crosses between two *Arabidopsis thaliana* accessions. A drawback with defining heterosis as increased values of quantitative measures rather than fitness components is that in a specific environment, an increased trait value is not necessarily associated with fitness. Heterosis may be a result of hybridization between populations with high levels of homozygosity (Rohde *et al.* 2004). This is because recessive deleterious alleles may become fixed within populations and interpopulation crosses can yield offspring with high levels of heterozygosity masking them.

The WISO Hypothesis

Parental conflict over resource allocation between seeds within a fruit after fertilization is stronger in outcrossing than in inbreeding populations. Brandvain and Haig (2005) developed

what they called the Weak Inbreeder/Strong Outbreeder (WISO) hypothesis, predicting that mechanisms for resource allocation to seeds that have evolved in response to parental conflict are stronger in populations with higher outcrossing rates. Selection on resource allocation to individual seeds should be strong in outcrossing populations given that seeds may be sired by different plants. In contrast, in highly selfing populations where most seeds are sired by the same plant, parental conflict over resource allocation should be relaxed. From their reasoning follows that the offspring fitness resulting from crosses between different population may be affected by outcrossing rates of the two populations and therefore the strength of selection on resource allocation in the parental populations.

The present study

I examined seed output and offspring fitness measured as size and growth rate after inter- (between-) and intra- (within-) population crosses in five European source populations of the alpine perennial herb *Arabis alpina*. Based on the assumption that weak outcrossing barriers have risen between distant populations, I tested the predictions that: (1) the number of seeds produced per fruit generated by crosses between plants from distant populations is lower compared to crosses between parents from the same source populations, (2) seed mass and germination rates are lower, and subsequent growth is slower in offspring generated by interpopulation crosses compared to offspring generated by intrapopulation crosses.

Materials and Methods

Study system

The divergence and interregional isolation of *A. alpina* in Europe offers an opportunity to investigate the effects of crosses between populations in an early state of differentiation. *Arabis alpina* is an alpine perennial polycarpic plant that has received considerable attention recently. There is an interest for establishing it as an alpine model organism because of its wide range and close relationship to the well-studied *A. thaliana* and *A. lyrata*. *Arabis alpina* has originally probably migrated from south-east into Europe (Koch *et al.* 2006) and current populations are re-colonisations from either glacial refugia in European or Middle Eastern regions following the last glacial period (Ehrich *et al.* 2007). Today, European populations are found in isolated or semi-isolated alpine regions. It has previously been demonstrated that the mating system varies among Italian populations from highly outcrossing to highly selfing (Ansell *et al.* 2008, Tedder *et al.* 2011). For self-compatible plants, spatial and temporal separation of anthers and stigma in are thought to reduce selfing (Medrano *et al.* 2005). Positive correlations between herkogamy (spatial separation of anther and stigma) and outcrossing have been found for some species (*e.g.* Motten and Stone 2000), but not for others (*e.g.* Medrano *et al.* 2005). For the populations in the present study, Wikström *et al.* (Unpublished) have found that herkogamy and anther orientation varies among the populations used in the present study and that herkogamy is negatively correlated to the rate of autogamous. Hence, natural populations may be expected to differ in outcrossing rates.

Plant material

In this experiment, I used populations of Iberian, Central European and Scandinavian origin. Plants originating from two Scandinavian (S1, S4), one French (F1) and two Spanish (E3, E4) populations were cross-pollinated by hand in a reciprocal design, producing interpopulation crosses. The number of plants per populations was between 15 and 25. Before pollination, flower buds chosen to receive pollen were marked with a colour-coded thread, indicating cross type (inter- or intrapopulation crosses). Experimental flowers were emasculated before petals had opened and stigmas were receptive to pollen. At opening, these flowers were pollinated with mature pollen by direct contact with anthers taken from their designated pollen donors. On each plant, each cross type was replicated three times whenever a sufficient number of flowers were available.

Seed output and germination

For each experimental flower, I counted and weighed the seeds produced. To examine seed quality, I scored the proportion of germinated seeds. Seeds were placed on filter papers in petri dishes that were constantly kept moist, at a temperature of 18°C and 18 h of light (long days). Due to differences in the number of replicates per cross type and number of seeds per fruit, the number of seeds whose germinability was tested varied from 10 to 54 per fruit. On four occasions over a period of 2½ weeks, germinated seedlings were counted and removed. The proportion of germinating seeds was estimated for each plant-cross combination as the mean proportion of seeds germinating among fruits examined.

Vegetative stage

Three randomly chosen newly germinated seedlings per fruit were planted into individual pots, and allowed to grow for five weeks in growth chambers at 18°C and days of 18 hour light. Rosette diameter of each plant was measured using a calliper and the numbers of leaves were counted on three occasions; 10, 17 and 34 days after plantation. On the last occasion, the numbers of above-ground shoots were also recorded.

Analyses

I calculated the mean of all trait values for fruits and offspring for each available combination of male and female parent. I used a separate ANOVA for each dam population to examine whether seed mass, the number of seeds per fruit and offspring plant diameter was affected by cross type. Sire population was treated as a fixed factor. I also tested whether the number of seeds per fruit after intrapopulation crosses varied among maternal populations using an ANOVA. To test whether germination rate and the number of shoots varied with paternal origin for plants grouped by maternal origin, Generalized Linear Models (GLMs) with a logit link and a quasibinomial distribution and log link and a quasipoisson, respectively, were evaluated by model comparison (*cf.* Crawley 2007). For effects on germination, a GLM with a logit link and a quasibinomial distribution was evaluated compared to a null-model. Tables were produced in Microsoft Excel 2010 and Notepad 6.1. For calculations, Microsoft Excel 2010 was used. R version 2.14.2 for Windows was used for all statistical analysis and R

version 2.14.2 for Windows and the package ggplot2 for R together with Microsoft Windows 7 Paint for production of figures.

Results

Seed characters

Pollen origin did not affect the number of seeds produced per fruit in inter- and intrapopulation crosses (Table 1, Appendix A).

Table 1. ANOVA statistics for variation in number of seeds per fruit owing to paternal origin for groups of dams after crosses within and among Arabis alpina populations from Scandinavia, France and Spain.

Dam	d. f.	F	p
E3	4	0.6899	0.6022
E4	3	1.8932	0.1499
F1	3	2.0609	0.1265
S1	4	0.8612	0.4942
S4	2	1.1005	0.3472

Among the intrapopulation crosses, the number of seeds produced per fruit differed between maternal populations ($df=4$, $F= 2.9722$, $p= 0.023$) and ranged from 26.0 ± 1.77 seeds per fruit in one Scandinavian (S1) to 34.1 ± 3.39 seeds per fruit in one Spanish (E4) population (Appendix A). Scandinavian plants produced heavier seeds after intrapopulation crosses than did plants from Spanish and French populations ($df= 4$, $F=3.253$, $p< 0.05$, Figure 1).

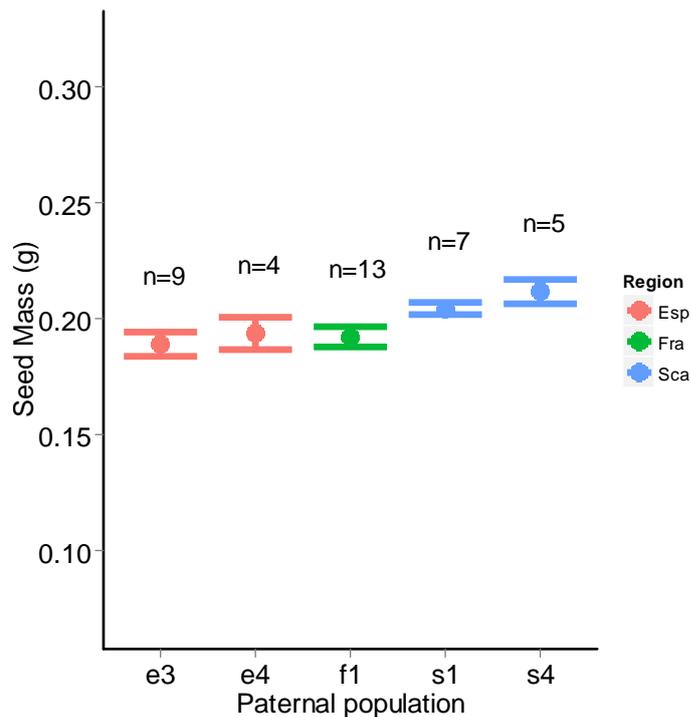


Figure 1: Seed mass after intrapopulation crosses of French, Scandinavian and Spanish plants. Maternal populations are depicted in top left corners. Means and SEM are indicated by point and brackets.

Both maternal and paternal origin affected seed mass. I could not detect outbreeding depression or heterosis among interpopulation crosses. Rather, the direction of the cross determined the direction of the effect (Figure 2a). Seeds sired by Scandinavian plants weighed less than did seeds sired by plants from the French or Spanish populations (Table 2, Figure 2b). Maternal plants produced lighter seeds when sired by pollen from Scandinavian populations than when sired by pollen from Spanish populations. The effects of sire origin on seed mass were largest for seeds produced by E3 dams, where E4 sired seeds with a mean mass 68% greater than S4 sires did. All crosses including French sires produced offspring of intermediate seed mass compared to those of Spanish and Scandinavian sires (Table 2). I could not find a correlation between mean seed mass and number of seeds per fruit (Pearson correlation, $df = 209$, $p = 0.1996$).

Table 2. ANOVA statistics for variation in seed mass owing to paternal origin for groups of dams after crosses within and among *Arabis alpina* populations from Scandinavia, France and Spain.

Dame	d. f.	F	p
E3	4	11.953	<0.001
E4	3	11.052	<0.001
F1	3	25.606	<0.001
S1	4	13.073	<0.001

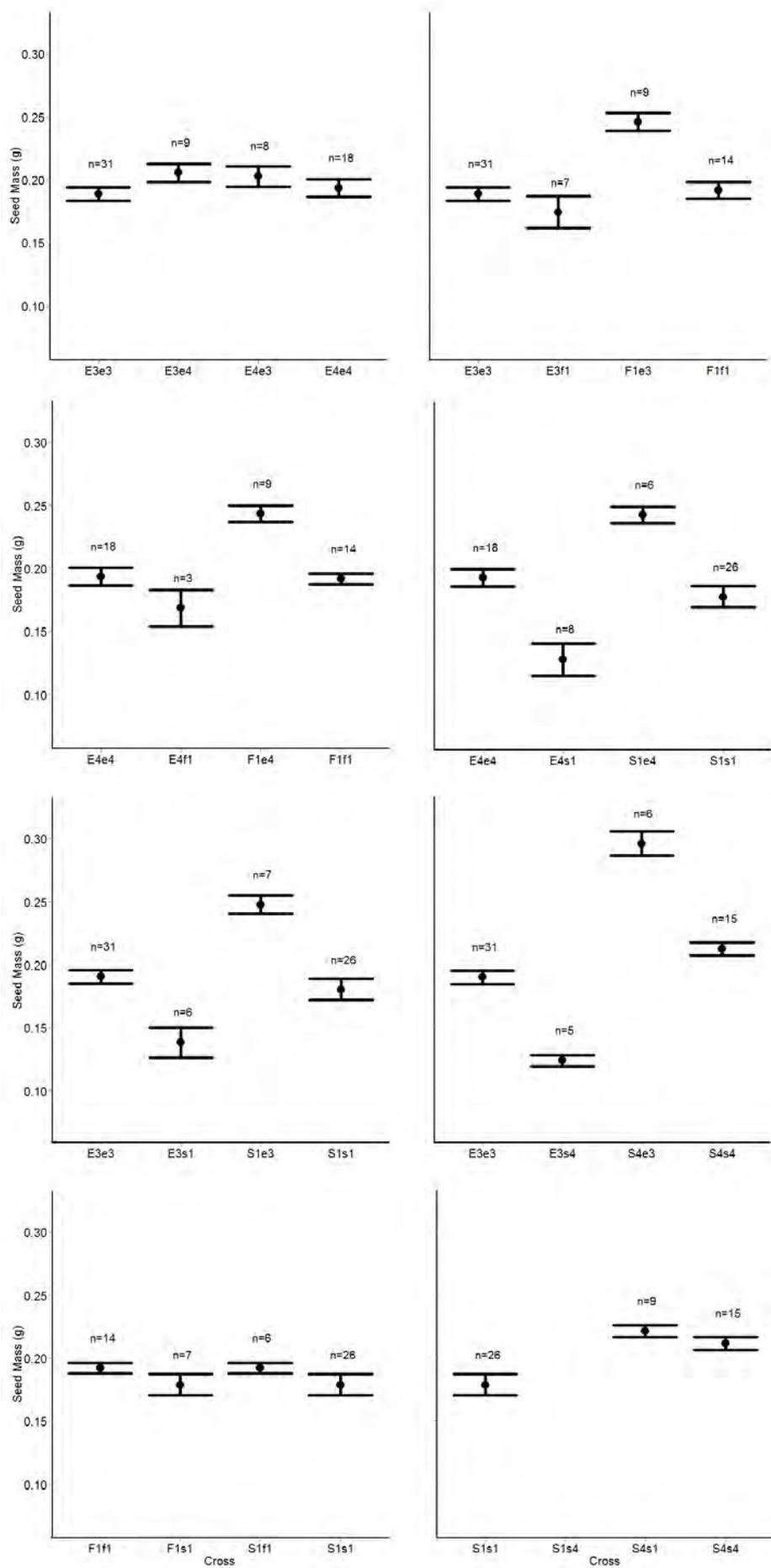


Figure 2a: Seed mass after crosses within and among *Arabis alpina* populations from Scandinavia, France and Spain. On the x-axis, capital letters denote maternal origin; lower case letters denote paternal origin. Means and SEM are indicated by point and brackets.

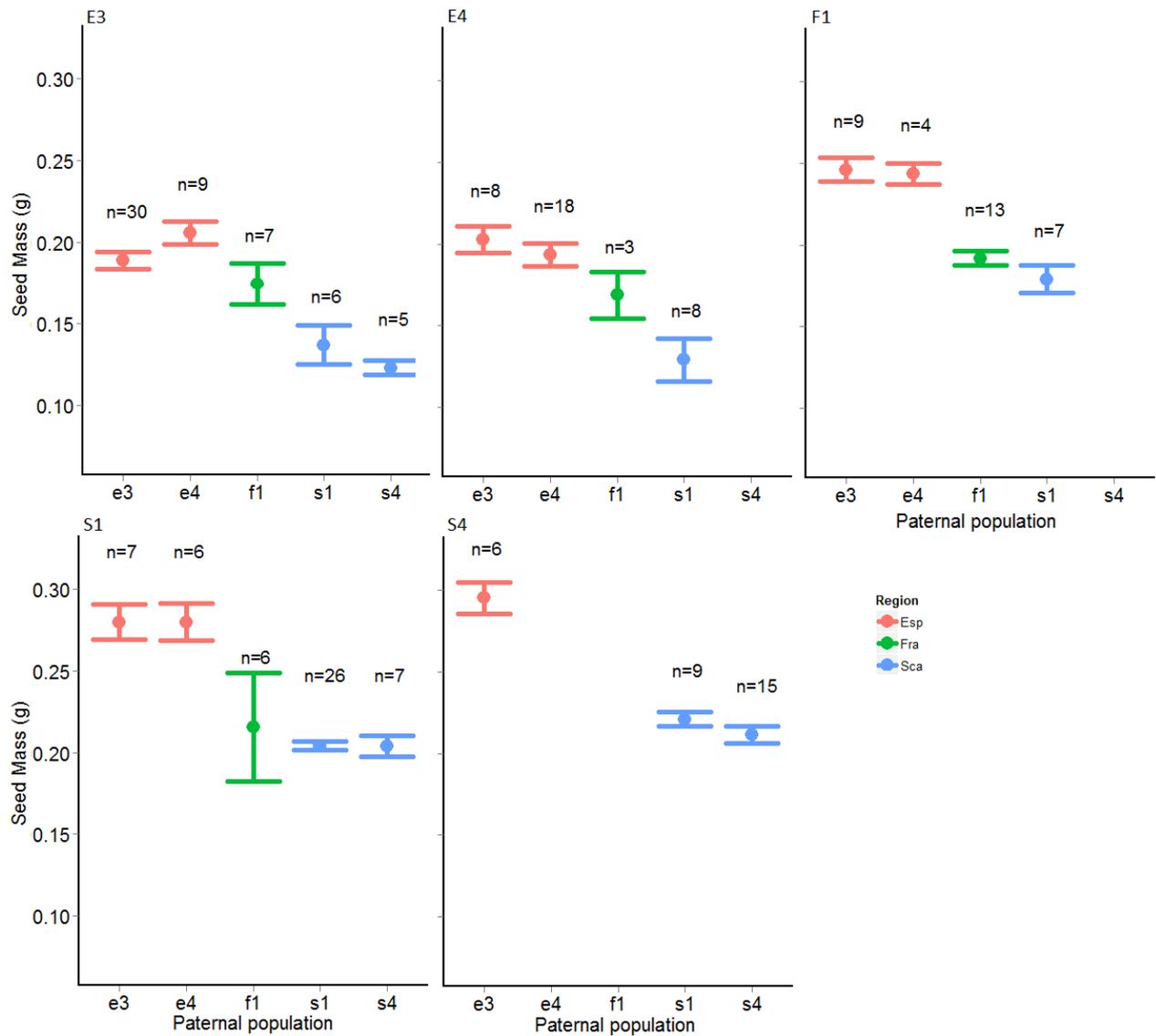


Figure 2b: Seed mass after crosses within and among *Arabis alpina* populations from Scandinavia, France and Spain. Maternal populations are depicted in top left corners. Means and SEM are indicated by point and brackets.

Mean seed mass did not significantly affect the mean proportions of seeds germinating (quasibinomial-based regression analysis, $df=99$, $F=1.2494$, $p=0.2664$). Paternal source population affected the proportion of the seeds that germinated for crosses descending from E4 and S4 mothers significantly (Figure 3). E4 dams produced seeds that were more germinable if pollinated by Spanish sires than if pollinated by Scandinavian sires ($df=33$, $F=5.5248$, $p<0.01$). Similarly, S4 dams produced seeds with higher germination rates when pollinated by Spanish than by Scandinavian sires ($df=29$, $F=4.2126$, $p<0.05$). Seeds from mothers of E3 and S1 origin displayed the same trend as E4 and S4, but differences were not significant.

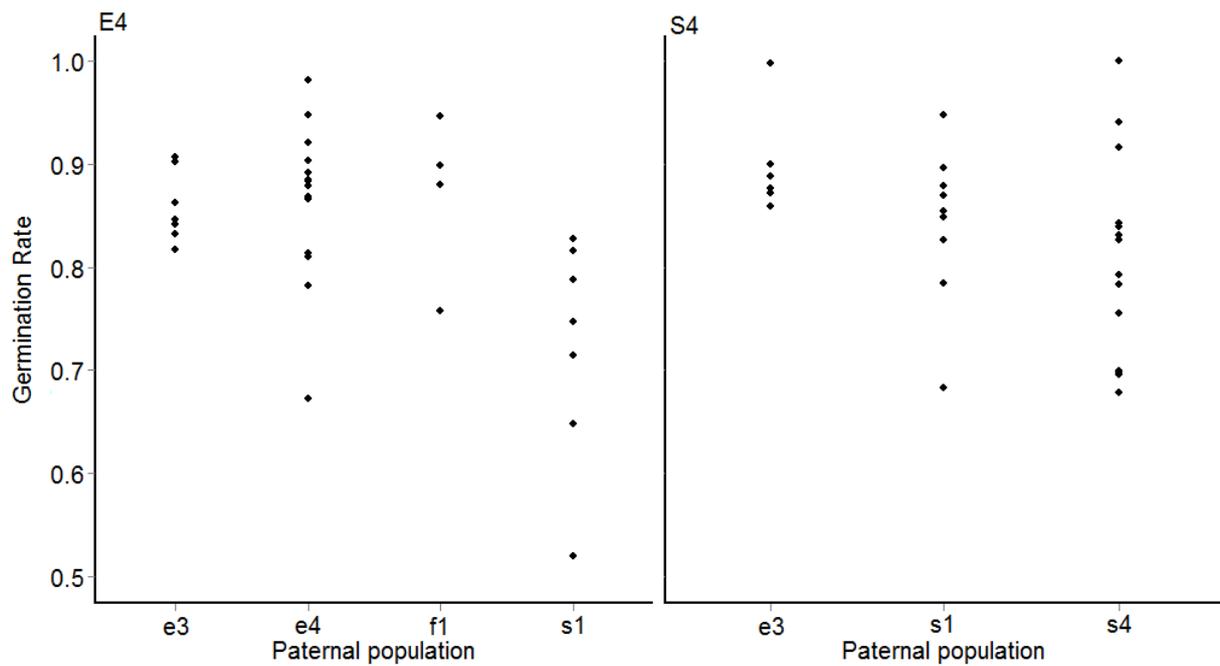


Figure 3: Germination Rates for seeds for offspring from inter- and intrapopulation crosses from Scandinavia, France and Spain. Maternal populations are depicted in top left corners. Data points represent 3 fruits from the same individual maternal plants.

Vegetative characters

For the three measures of size (rosette diameter, shoot- and leaf number), offspring resulting from crosses with sires from Spanish populations were in some of the recordings larger than offspring with Scandinavian sires. Seed mass was strongly positively correlated to rosette diameter at the first ($r=0.299$) and second ($r=0.308$) recording ($df=200$, $p>0.001$ for both correlations). Scandinavian dams also produced offspring with wider rosette diameters after 10 and 17 days when crossed with Spanish than with Scandinavian sires (Table 3, Figure 4), but there were no statistically significant differences after 34 days (Table 3, Figure 4). Offspring of French dams also had a wider diameter after 17 days if sired with Spanish plants than if sired by Scandinavian plants (Table 3, Figure 5).

Table 3. ANOVA statistics for variation in diameter owing to paternal origin for groups of dams 10, 17 and 34 days after sowing after crosses within and among Arabis alpina populations from Scandinavia, France and Spain.

Dame	Scoring	d. f.	F	p
E3	1st	4	0.7728	0.5478
	2nd	4	1.1919	0.3251
	3rd	4	1.6481	0.1784
E4	1st	3	1.2683	0.303
	2nd	3	1.6878	0.1907
	3rd	3	0.5694	0.6393
F1	1st	3	2.0816	0.1244
	2nd	3	3.4188	<0.05
	3rd	3	0.7523	0.528
S1	1st	4	3.6327	<0.05
	2nd	4	6.1692	<0.001
	3rd	4	1.4356	0.237
S4	1st	2	5.1765	<0.05
	2nd	2	10.869	<0.001
	3rd	2	2.6173	0.09142

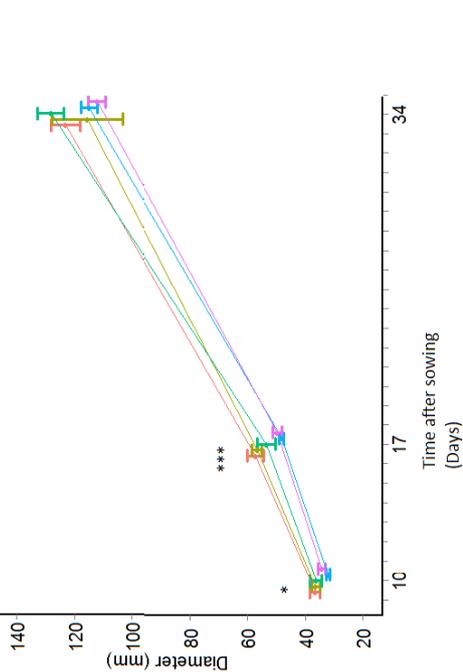
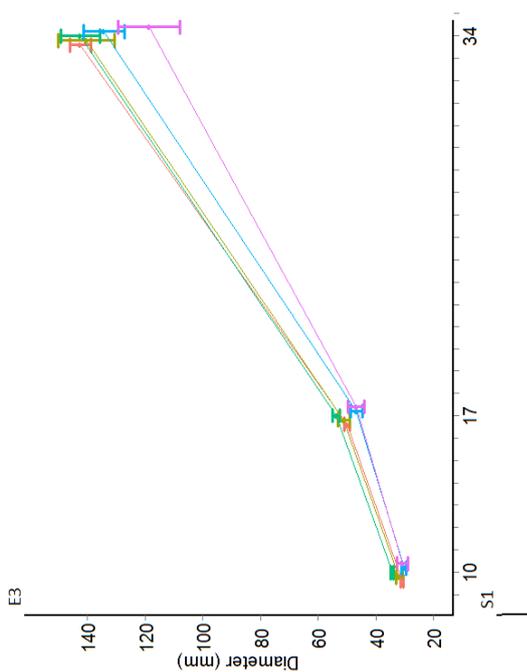
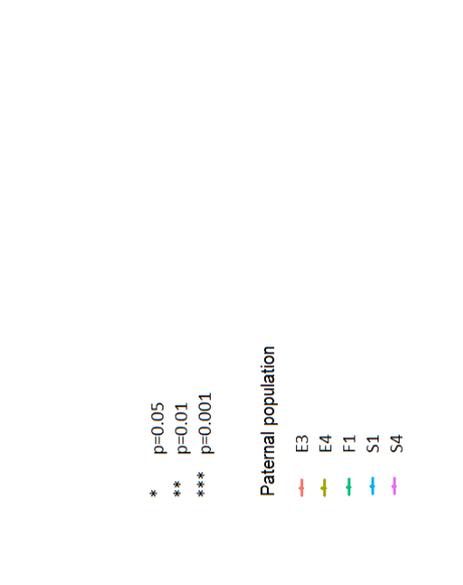
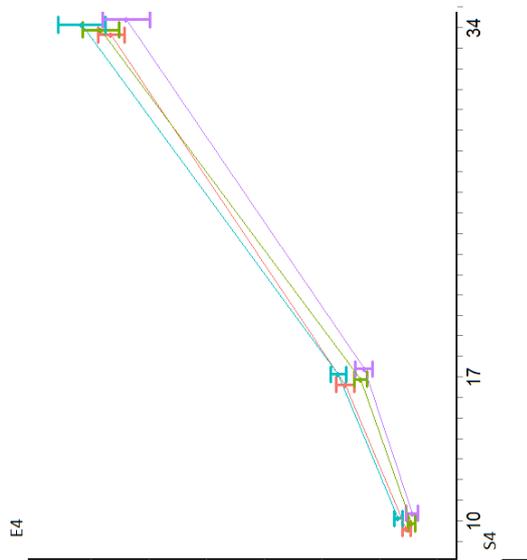
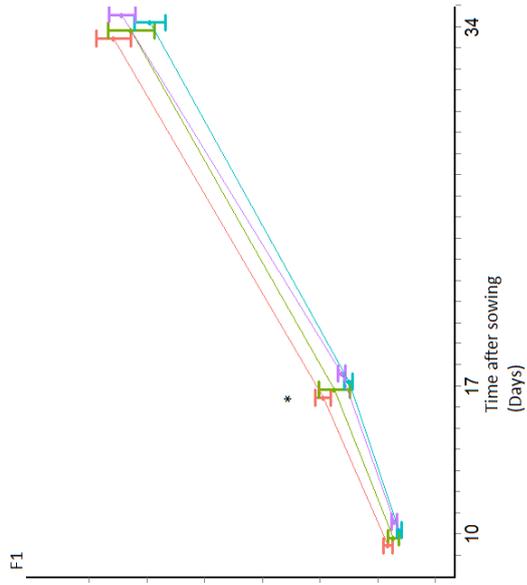


Figure 4: Rosette diameter 10, 17 and 34 days after sowing for offspring from crosses within and between *Arabis alpina* populations from Scandinavia, France and Spain. Maternal origin depicted in top left corners. Means and SEM are indicated by point and brackets.

Maternal plants from the Scandinavian populations (S1, S4) and from one Spanish (E3) population produced offspring with more vegetative shoots when crossed with Spanish or French sires than when crossed with Scandinavian sires (Table 4, Figure 5). Effects of cross type on number of leaves produced was observed only for offspring of S1 dams after 10 days (GLM, $df=51$, $F= 4.3148$, $p< 0.01$) and 17 days (GLM, $df=206$, $F= 4.4637$, $p< 0.01$), when offspring of Spanish pollen donors were larger than those of pollen donors from Scandinavian populations.

Table 4. ANOVA statistics for variation in number of shoot per individual owing to paternal origin for groups of dams after crosses within and among *Arabis alpina* populations from Scandinavia, France and Spain.

Dame	d. f.	F	p
E3	57	3.4164	0.01473
E4	35	0.3745	0.7719
F1	32	1.3172	0.2878
S1	51	5.648	<0.001
S4	29	10.178	<0.001

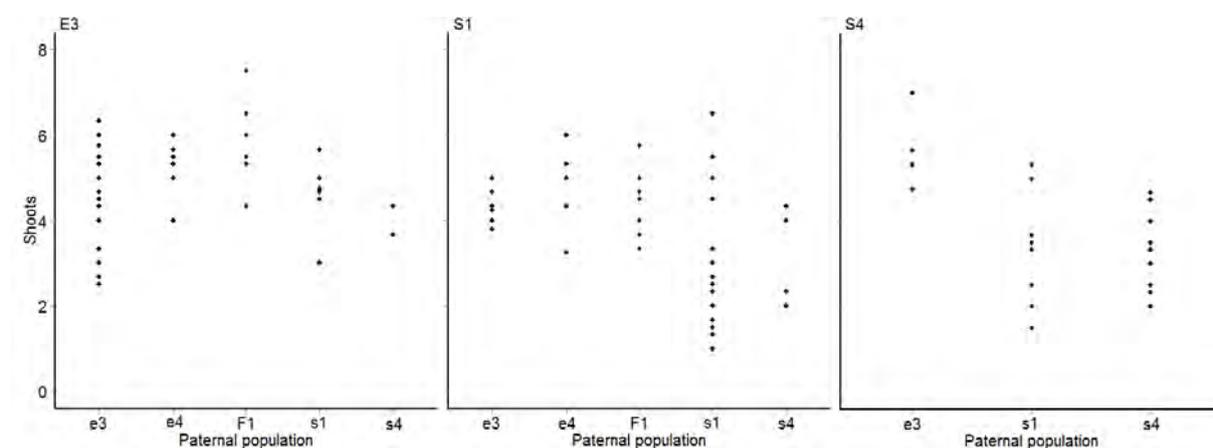


Figure 5: Numbers of shoots produced by plants for offspring from inter- and intrapopulation crosses from Scandinavia, France and Spain. Maternal genotype origins are depicted in top left corners. Data points represent 3 individual plants in the F1 generation resulting from one maternal plant.

Discussion

Although the continental and Scandinavian lineages of *A. alpina* in this experiment have probably diverged only since the last ice age, the present study demonstrates that there is population differentiation giving paternal and maternal effects on seed characters and early growth rate. The most striking result was that both parental genotypes affected seed mass to a large extent. In general, seed mass was higher among seeds with Spanish paternal origin and lower among seeds with Scandinavian paternal origin. In contrast, maternal origin had inverse effects. That is; seed mass was lower for seeds from Spanish mothers than for seeds from Scandinavian mothers. French paternal and maternal effects were intermediate to those of Spanish and Scandinavian parental effects. The results of this study are consistent with neither of the two predictions stated in the introduction; (1) Seed production was not lower in crosses between distant populations and (2) seed mass, germination and growth were not uniformly reduced in crosses between distant populations.

Seed mass

This study demonstrates that both maternal and paternal genotype affect seed size in *A. alpina*. Interregional crosses gave higher seed mass than intraregional crosses for Scandinavian, but not Spanish plants. It has previously been demonstrated that seed mass is affected by maternal genotype in *A. thaliana* (Fang *et al.* 2012), and it seems to be the case also for *A. alpina*, given comparisons of seed mass of intrapopulation crosses in this experiment. Other studies have indicated that paternal effects may also influence seed size in *A. thaliana* (House *et al.* 2010, de Jong and Scott 2007, 2005). Moreover, previous studies of other species have found paternal effects on embryo size (Diggle *et al.* 2010), seed size (*e.g.* Andersson 1990, Diggle *et al.* 2010) and seed mass (Richardson and Stephenson 1991), as well as plant size (Marshall and Whittaker 1989).

The WISO hypothesis

The WISO hypothesis predicts that when different alleles are being favoured in populations with different outcrossing rates hybrids from crosses between these populations can show parent-of-origin effects (Brandvain and Haig 2005), effects that differ depending on parent sex. Haig (2000) has argued that a parental conflict in resource allocation to seeds within a fruit may be explained by a model based on Hamilton's rule (*cf.* Hamilton 1964 I, II). That is; we may expect individual seeds to enhance resource allocation to themselves at the expense of allocation to other seeds. An allele with such parental conflict-effects should be selected for if it is more beneficial for the fitness of one sex than it is disadvantageous for the other (Haig 1997). Additionally, selection pressure for such alleles should be weaker in selfing populations because most seeds produced by a dam will have the same sire. In a later study by Brandvain and Haig (2005), the authors developed this line of thought and proposed the WISO hypothesis, predicting that by crossing plants from outcrossing and inbreeding populations, one would produce offspring with parent-specific origin effects. Since resource allocation between seeds within fruits is not critical for paternal fitness in inbreeding

populations, offspring from crosses between outcrossing and inbreeding populations may be expected to gain resources biased towards the outcrossing parent (Brandvain and Haig 2005). Interpopulation differences in mating systems would make the WISO hypothesis applicable for results from the present experiment if the Scandinavian populations have lower outcrossing rates than Spanish populations. Greenhouse and field experiments and floral morphology (herkogamy and anther orientation) suggests that selfing rates are higher in the Scandinavian than in French and Spanish populations (Wikström *et al.*, unpublished). As interpopulation differences in outcrossing rates have been found among other natural self-compatible populations of *A. alpina* (Tedder *et al.* 2011), it is not unlikely that variation in outcrossing rate also exists among the populations used in the present study.

However, to definitely conclude that my results are in line with the WISO hypothesis, estimations of outcrossing frequencies in the source populations would be needed. An alternative explanation to my results may be that different strengths of parental conflict in the populations are also a product of different life history strategies, where resource allocation towards overall seasonal higher seed production is promoted in Spanish sires but not in Scandinavian counterparts. Another possible explanation to pollen origin effects on offspring seed mass could be lower genetic variation in Scandinavia because of a founder effect as a result of historically small population sizes.

Seed number

Seed number is an important fitness component and differences between offspring produced by controlled crosses have been found in other study systems (*e.g.* *A. thaliana*, House *et al.* 2010). In my study, however, no differences in seed production could be detected among any crosses. House *et al.* (2010) found a significant effect of paternal origin for seed number in *A. thaliana*, and in that example fruit position on the plant was controlled for in the experiment. Since fruit position was not taken into account in this experiment, eventual differences may have been missed.

In this study, no correlation between seed mass and number was detected. Given that a maternal plant has limited resources to allocate to reproduction, one could expect a trade-off between producing many and high-quality seeds. Andersson (1990) found a trade-off between seed weight and number in *C. tectorum*. However, the lack of correlation between seed number and mass for fruits concurs with the results of de Jong *et al.* (2011), where *A. thaliana* was used in a crossing experiment. The slope of the negative correlation found in *C. tectorum* by Andersson (1990) was not steep, indicating that possible differences may be hard to detect. Thus, larger sample sizes may be necessary to detect such an association, would it exist in *A. alpina* or other members of the Brassicaceae family. It could also be helpful to somehow control for variation in resource acquisition ability.

Germination

Germination success for crosses involving E4 or S4 dams depended on origin of source population for sires. In both cases, Spanish fathers produced seeds with a higher germination rate than Scandinavian fathers did. The finding of paternal effects on germinability agrees

with observations in intraspecific crosses of *A. thaliana* (House *et al.* 2010) and interspecific crosses of *Silene vulgaris* and *Silene uniflora* (Andersson *et al.* 2008). The absence of a relationship between seed mass and germination success among seeds from intrapopulation crosses may be due to highly favourable germination conditions in the lab. A similar analysis was not made with the data on interpopulation crosses as the groups were heterogeneous with respect to parental origin. Although I could not find an association between seed mass and germination for intrapopulation crosses in this experiment, studies of other species have found that non-germinated seeds are smaller than seeds that do germinate (*e.g.* Andersson 1990, 1996) and that seedling survival over time can increase with seed size under harsh environmental conditions (Krannitz *et al.* 1991). It would be worthwhile to perform a germination experiment, in a set of different conditions including different temperatures and moistures, or in natural conditions at the sites of the five study populations to control for condition-sensitive germination success.

Vegetative growth

The higher initial growth rate among Scandinavian plants crossed with Spanish and French plants compared to those pollinated with Scandinavian pollen may be interpreted as paternal effects on early growth independently or as large seeds allowing seedlings to develop faster. Significant correlations between seed mass and early rosette size have previously been found in studies of *Arabidopsis thaliana* (El-Lithy *et al.* 2004) and *Centaurea maculosa* (Weiner *et al.* 1997). There was a strong positive correlation between seed mass and these early diameter measurements in the present study, indicating that seed mass is an important determinant for early growth. Higher early growth rates may be an advantage in establishment, and overall size is often strongly positively correlated to plant fitness. For polycarpic plants, the resources spent on flowering are often involved in a trade-off with resources stored for future reproduction (Brys *et al.* 2011).

Conclusions

In this study, I detected strong paternal effects on seed mass in all populations. In all cases, Spanish sires fathered the heaviest seeds and Scandinavian sires the lightest. Overall, Scandinavian dams produced heavier seeds and initially faster growing plants if crossed with continental plants. Hence, both weak post-zygotic crossing barriers in the form of lower seed mass (for crosses involving Spanish dams) and an enhancing effect on seed mass and early growth (for crosses involving Scandinavian dams) were found in interregional crosses. This pattern shows no sign of heterosis since enhancing effects were unidirectional for crosses. These results may instead be explained by differences in mating systems leading to paternal conflicts being stronger in populations with higher outcrossing rates, supporting the WISO hypothesis formulated by Brandvain and Haig (2005). Therefore outcrossing rates should be estimated and variation in seed weight within a fruit due to different paternal origin should be quantified.

Further work

For the populations of *A. alpina* included in this study and other in from the same geographical regions, genotyping is underway. Genotyping may reveal differences in outcrossing rates in the study populations and will thus allow an evaluation of parental conflict and the WISO hypothesis as explanations to parts of the observed variation in seed mass. The populations have recently been shown to exhibit differences in floral morphology and autogamous seed production (Wikström *et al.*, unpublished), possibly indicating differentiation in mating systems. It would be interesting to further investigate differentiation and to study mating systems by performing inter- and intrapopulation crosses between populations over yet greater distances than in the present study to cover a wider range of populations with additional variation in mating system. Preliminary results of interpopulation crosses between Anatolian (ssp. *caucacea*), Swedish and Spanish (E3) plants show patterns of asymmetrical seed development; the Anatolian self-incompatible plants produced no seeds when treated with pollen from Spanish or Scandinavian plants (Toräng *et al.*, unpublished). In contrast, Spanish and Scandinavian plants produced many small seeds when sired by Anatolian plants (Toräng *et al.*, unpublished). Besides expanding the geographical range, the ecological importance of seed mass, flowering phenology and mating system in *A. alpina* should be investigated in field studies. A reciprocal transplant experiment, also including crosses, adjacent to natural populations of known relative outcrossing rates should help to disentangle variables determining patterns of paternal effects seen in my study.

Acknowledgements

I would like to thank my supervisors, Per Toräng and Jon Ågren, for guidance, support and feedback, Mattias Vass and Linus Wikström for assistance and stimulating discussions, and opponents Beata Edström and Camille Madec for feedback on the written thesis.

References

- Andersson S. Månsby E. Prentice HC. 2008. Paternal effects on seed germination: a barrier to the genetic assimilation of an endemic plant taxon?. *Journal of Evolutionary Biology*. 21: 1408-1417
- Andersson S. 1996. Seed size as a determinant of germination rate in *Crepis tectorum* (Asteraceae): evidence from a seed burial experiment. *Canadian Journal of Botany* 74:568-572
- Andersson S. 1990. Effects on Seed Size in a Population of *Crepis tectorum* (Asteraceae). *Oikos* 59: 3-8
- Barth S. Busimi AK. Friedrich Utz H. Melchinger AE. 2003. Heterosis for biomass yield and related traits in five hybrids of *Arabidopsis thaliana* L. Heynh. *Heredity* 91: 36-42
- Birchler JA. Yao h Chudalayandi S. Vaiman D. Veitia RA. 2010. Heterosis. *The Plant Cell* 22:2105-2112

- Bomblies K. 2010. Doomed Lovers: Mechanisms of Isolation and Incompatibility in Plants. *Annual Review of Plant Biology* 61: 109-124
- Brys R. Shefferson RP. Jacquemyn H. 2011. Impact of intrinsic and extrinsic variables on flowering and reproductive allocation patterns in a perennial iteroparous grassland herb: a ten-year experiment. *Oecologia* 166:293-303
- Coyne J. Orr HA. 2004. *Speciation*. 1st ed. Sinauer Associates. Sunderland
- Crawley MJ. 2007. *The R Book*. John Wiley & Sons Ltd. Chichester
- Diggle PK. Abrahamson NJ. Baker RL. Barnes MG. Koontz TL. Lay CR. Medeiros JS.
- Ehrich D. Gaudeul M. Assefa A. Koch MA. 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* 16:2542-2559
- El-Lithy ME. Clrekx EJM. Ruys GJ. Koornneef M. Vreugdenhil D. 2004. Quantitative Trait Locus Analysis of Growth-Related Traits in a New Arabidopsis Recombinant Inbred Population. *Plant Physiology* 135:444-458
- Etterson JR. Keller SR. Galloway LF. 2007. Epistatic and Cytonuclear Interactions Govern Outbreeding Depression in the Autotetraploid *Campanulastrum americanum*. *Evolution* 61: 2671-2683
- In press: Fang W. Wang Z. Rongfeng C. Li J. Li Y. Maternal control of seed size by EOD/CYP78A6 in *Arabidopsis thaliana*. *The Plant Journal* . doi: 10.1111/j.1365-313X.2012.04907.x
- Hamilton WD. 1964 The Genetical Evolution of Social Behaviour I. *Journal of Theoretical Biology* 7: 1-16
- Hamilton WD. 1964 The Genetical Evolution of Social Behaviour I. *Journal of Theoretical Biology* 7: 17-52
- Haig D. 1997. Parental antagonism, relatedness asymmetries, and genomic imprinting. *Proceedings of the Royal Society of London B* 264:1657-1662
- Haig D. 2000. The kinship theory of genomic imprinting. *Annual Reviews of Ecology and Systematics* 31:9-32
- House C. Roth C. Hunt J. Kover PX. 2010. Paternal effects in *Arabidopsis* indicate that offspring can influence their own size. *Proceedings of the Royal Society*. 277: 2885-2893
- de Jong TJ. Hermans CJ. van der Veen-van Wijk CAM. 2011 Paternal effects on seed mass in *Arabidopsis thaliana*. *Plant Biology* 31:71-77
- de Jong T. Scott RJ. 2007. Parental conflict does not necessarily lead to the evolution of imprinting. *Trends in Plant Science* 12: 439-443

- de Jong TJ, van Dijk H, Klinkhamer PGL. 2005. Hamilton's rule, imprinting and parent-offspring conflict over seed mass in partially selfing plants. *Journal of Evolutionary Biology*. 18: 676-682
- Koch MA, Kiefer CK, Ehrich D, Vogel J, Brochmann C, Mummenhoff K. 2006. Three times out of Asia Minor: the phylogeography of *Arabis alpina* L. (Brassicaceae). *Molecular Ecology* 15:825-839
- Krannitz PG, Aarssen LW, Dow JM. 1991. The Effect of Genetically Based Differences in Seed Size on Seedling Survival in *Arabidopsis thaliana* (Brassicaceae). *American Journal of Botany* 78: 446-450
- Lamb EM, Davis DW, Andow Da. 1993 Mid-parent heterosis and combining ability of European corn borer resistance in maize. *Euphytica* 72: 65-72
- Leinonen PH, Remington DL, Savolainen O. 2011. Local adaptation, phenotypic differentiation, and hybrid fitness in divergent natural populations of *Arabidopsis lyrata*. *Evolution* 65: 90-107
- Marshall DL. 1989. Whittaker KL. Effects of Pollen Donor Identity on Offspring Quality in Wild Radish, *Raphanus sativus*. *American Journal of Botany* 76: 1081-1088
- Medrano M, Herrera CM, Barret SCH. 2005 Herkogamy and Mating Patterns in the Self-compatible Daffodil *Narcissus longispathus*. *Annals of Botany* 95:1105-1111
- Richardson TE, Stephenson AG. 1991. Effects of Parentage, Prior Fruit Set and Pollen Load on Fruit and Seed Production in *Campanula Americana* L.. *Oecologia* 87: 80-85
- Rohde P, Hicha DK, Heyer AG. 2004. Heterosis in the freezing tolerance of crosses between two *Arabidopsis thaliana* accessions (Columbia-0 and C24) that show differences in non-acclimated and acclimated freezing tolerance. *The Plant Journal* 38: 790-799
- Tedder A, Ansell SW, Lao X, Vogel JC, Mable BK. 2011. Sporophytic incompatibility genes and mating system variation in *Arabis alpina*. *Annals of Botany* 108: 699-713
- Weiner J, Martinez S, Müller-Schärer H, Stoll P, Schmid B. 1997. How important are environmental maternal effects in plants? A study with *Centaurea maculosa*. *Journal of Ecology* 85:133-142
- Widmer A, Lexer C, Cozzolino S, 2009, Evolution of reproductive isolation in plants, *Heredity* 102:31-38