EVIDENCE OF POLLINATOR-MEDIATED SELECTION FOR FLORAL DISPLAY HEIGHT

EVIDENCE OF POLLINATOR-MEDIATED SELECTION FOR FLORAL DISPLAY HEIGHT

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ABSTRACT

Many floral characters have can affect plant fitness by altering the frequency or efficiency of pollinator visits. More frequent pollinator visitation may lead to larger pollen loads and greater outcrossing rates, which in tern may lead to greater numbers of offspring, with higher genetic variability and less inbreeding depression. Mating systems may moderate the strength of natural selection on traits that attract pollinators through its effects on inbreeding depression, and the ability to self-pollinate autogamously. Natural selection is expected to be stronger in largely outcrossing systems where inbreeding depression is high, but reduced in largely selfing systems where deleterious alleles have been purged.

Here I investigate the role of floral display height in attracting pollinators, and its consequences for plant fitness in two closely related monocarpic herbs (*Hesperis matronalis* and *Alliaria petiolata*) with different mating systems. Though others have looked at height as a potential trait attracting pollinators, no one has yet clearly separated the effects of floral display height from that of plant size. I experimentally altered plant height independently of plant size by placing potted plants into either elevated or non-elevated treatments and observed pollinator foraging behaviour and reproduction.

Pollinators preferred taller floral displays in the predominant outcrosser *Hesperis matronalis*. Elevated plants produced more seeds than control plants although their seeds were smaller, reflecting a strong seed size-number trade-off. Path analysis showed seed number was affected directly by plant height, presumably though greater pollinator visitation, and indirectly by plant mass through increased fruit number.

In *Alliaria petiolata*, a predominant self-pollinating species, pollinators visited taller displays in field observations and experimentally elevated plants had significantly greater seed per fruit. Experimentally outcrossed and self-pollinated plants showed no difference in seed production, but offspring quality was significantly higher in outcrossed plants for early traits. Measures of maternal fitness increased significantly with both maternal and seed mass. For individual seeds, seed mass was the most important factor in seed fitness, where larger seeds germinated sooner, more frequently, and had higher survivorship than smaller seeds, regardless of pollination treatment. Competition strongly affected all seedling traits, with increased density having a significant negative effect on seedling mass, total leaf area, and maximum leaf length; and, a positive effect on hypocotyl length and petiole length. Seedling mass at harvest was not significantly affected by elevation treatment, seed mass, or maternal mass, however, this may have been due to lower competition brought about by low germination and survival; thus, competition did not accentuate the effect of maternal size or elevation treatment on seedling. Taken together, the results of these two studies suggest the importance of height, in attracting pollinators, independent of size in both species. The consequences of pollinator attraction were highly significant in the predominant outcrosser *Hesperis matronalis*, while maternal size and seed size played more important roles in the fitness of the predominant selfer *Alliaria petiolata*

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Floral display height

The majority of flowering plants require animal vectors for successful pollination. Enhancing a plant's ability to recruit pollinators increases the likelihood of pollen receipt by stigmas, thus increasing the potential for cross-fertilization. The frequency and/or efficiency of pollinator visits can be affected by a variety of traits, such as flower colour (Delph and Lively 1989, Spaethe et al. 2001) size (Schemske and Bradshaw 1999), morphological symmetry (Moller and Eriksson 1995), and floral display size (Robertson and Macnair 1995). Few studies, however, have looked at the effect of floral display height as a possible trait in pollinator attraction (Schaffer and Schaffer 1979, Donnelly et al. 1998, Gumbert and Kunze 1999, Peakall and Handel 2003).

Floral display height may affect pollinator visitation for several reasons. Higher floral displays may simply increase the area from which a potential pollinator may spot it (Roubik 1993). For plant species that grow in dense stands, the surrounding vegetation may hide shorter floral displays; taller floral displays emerging above the stand may be more visible to passing pollinators from a greater distance. Manoeuvrability between flowers may also explain a preference for taller floral displays. Because short floral displays often reside in dense vegetation, manoeuvring between flowers may incur a significant cost to flight and time per unit of food reward (Gumbert and Kunze 1999, Wolf et al. 1999). Taller floral displays above the main vegetative mass allow for more direct and efficient travel between floral displays (Roubik 1993). Another possible explanation for the importance of floral display height may lie in the strong correlation between floral display height and plant size. Since floral display height is partly determined by increased inter-nodal lengths, it is strongly correlated with plant size. If larger plants produce more or higher quality food (i.e. pollen and nectar) due to their greater access to resources, then floral display height may simply act as a cue by which a pollinator can quickly assess a potential food source from afar (Wells et al. 1992, Dreisig 1995). Thus, pollinators may visit taller floral displays more frequently because they provide larger food rewards than floral displays of a smaller plants, increasing efficiency under optimal foraging theory (Schaffer and Schaffer 1979, Larson and Larson 1990). These possibilities are not mutually exclusive and all or some may be occurring together.

Regardless of the mechanism underlying pollinator attraction, increased floral display height may promote both female and male fitness: female fitness would be increased by producing a higher ratio of outcrossed to selfed seed and by reducing pollen limitation, thus producing more seeds; male fitness would benefit by increasing pollen export by pollinators and, thereby, increased reproduction (Galen 1992). Because plant size and height are strongly correlated, I experimentally altered plant height independent of plant size to better understand the role of floral display height in pollinator attraction.

Increased height can alter fitness through many mechanisms other than its effects on pollinators. Stem elongation may play multiple roles, as it also allows for increased leaf area without crowding and is important in competition for carbon acquisition in dense stands (Dudley and Schmitt 1996, Sanchez 2002). Plant height may also play a role in temperature regulation: leaves on taller stems allow plants to lower their temperature in hot climates; and short rosettes, with leaves close to the ground, allow plants to maintain or gain heat in cold climates (Givnish 1982). Furthermore, taller stems may provide developed seeds a wider range for dispersal, as seeds fall off the mother plant (Klinkhamer and De Jong 1983, Stokes and Yeaton 1995). Dispersal from taller stems may also reduce density dependent predation and/or sibling competition (Harper 1977). These possible roles for taller stems are not mutually exclusive, and it is likely that some or all are playing a part in increasing plant fitness.

Floral display height is likely to be strongly determined by environmental factors because it is so closely linked to plant size, unlike flower colour and shape, which are known to be heritable, and are less likely to be affected by plant size. The extent to which plant size is genetically determined is unclear (Gottlieb 1977). Since floral display height is linked to plant size, it is logical to assume that any genetic component for plant height would be affected by variations in both the physical and biological environment. Nutrient availability, light levels, and moisture content all may affect plant size, while density can cause stem elongation and thus raise floral display height (Dudley and Schmitt 1996).

The strong association between plant height and plant size makes it difficult to separate any benefits of height in pollinator attraction from the many benefits of increased plant size. In this thesis, I hypothesize that when height is manipulated artificially, shorter floral displays will produce a higher ratio of selfed to outcrossed seeds than their taller counterparts due to differences in pollinator visitation frequencies and, thus, produce offspring of lesser quality.

1.2 Mating systems

Selection on floral traits that increase pollination should be affected by a plant's mating system, because the importance of pollinators to seed set and seed quality varies with mating system. Mating systems run the spectrum, from predominant outcrossers to predominant selfers (Vogler and Kalisz 2001). Outcrossing species rely heavily on pollinators (unless wind pollinated) for successful transfer of pollen and, thus, experience strong selection for floral traits that increase pollinator visitation and efficacy (Stebbins 1970). Among species in this group, one expects to find vast variation in floral traits, which have been moulded by selection pressures to suit specific pollinators.

In many species, outcrossing is always favourable, though not always possible (Schemske and Lande 1985). When pollinator visitation is low, however, self-pollination can assure reproduction. Predominantly outcrossing species that experience reduced visitation by pollinators may experience reduced fitness due to reduced seed set (fecundity) or reduced seed quality if the plant increases the ratio of selfed to outcrossed seed (Schemske and Lande 1985). In predominant outcrossing species, selfing comes with a higher cost to fitness, as these species often experience high rates of inbreeding depression (Charlesworth and Charlesworth 1987). Husband and Schemske (1996), demonstrated that recessive lethal and highly deleterious alleles are important factors in inbreeding depression in predominant outcrossers.

Predominantly self-pollinating species are not as dependent on pollinators to bring or take pollen. In such species, pollinator visitation frequency may not affect seed set since self-pollen is readily available and inbreeding depression may be low due to a history of purging of deleterious alleles (Husband and Schemske 1996). However, predominant selfers may also experience lower fitness when selfed due to the persistence of mildly deleterious alleles, which may not be purged by selection (Charlesworth et al. 1990a). For predominant selfers, reduced fitness may not be evident until later stages in the life cycle (Schmitt and Ehrhardt 1990). Thus, a plant's mating systems will determine the magnitude of reduced fitness due to inbreeding and where it will be expressed in the life cycle (Husband and Schemske 1996).

1.3 Floral trait differences between outcrossing and selfing species

Predominant outcrossers often show strong directional selection on the floral traits as compared to selfers (Johnson and Steiner 2000). Thus, predominant outcrossers can be generalized in comparison to selfers as having larger, showier flowers, producing more nectar, often having deeper corollas, and exhibiting a greater separation of stamens and stigmas (Faegri and van der Pijl 1979). Such traits increase plant fitness by a combination of increased pollinator visitation, pollinator exclusivity, or pollinator efficacy (Grant 1994). Increasing pollinator visitations may increase deposition of intraspecies pollen to the stigma, whereas the shape, size, colour, and orientation may increase the efficiency of a pollinator's ability to deposit pollen. Increased pollinator visits can in some cases lead to greater self-pollination through geitonogamy. If a plant has many flowers open at the same time, a pollinator may visit several or all consecutively, thus pollinating some flowers with pollen from others of the same plant.

Pollinator exclusivity means that a plant's floral traits exclude some potential pollinators to the advantage of others. By reducing the number of visiting pollinator species, a plant is more likely to receive pollen from another individual of the same species rather than foreign pollen that may interfere with future fertilization (Tremblay 1992). Thus, predominant outcrossers tend to have fewer species of pollinators, but such pollinators tend to visit few species of flowers, increasing the chances of the right pollen grain landing on the right stigma. In contrast, predominant self-pollinating species tend toward more general flower shapes with stamens and stigmas closely situated, promoting self pollination, with fewer rewards, and higher pollinator diversity (Johnson and Steiner 2000).

1.4 Biennial and monocarpic perennial life histories and plant architecture

Monocarpic (= semelparous) species experience a single reproductive event after which the plant dies. Such species have been called "Big Bang producers" to highlight their post reproduction mortality as a result of transferring all resources to reproduction at the cost of continued vegetative growth (Gadgil and Bossert 1970). Most monocarpic species are annuals but some have evolved a specialized life history known as monocarpic perennial. These are species in which reproduction is delayed to the second or later years based on some minimum plant size. A special case is the obligate biennial, in which reproduction is induced in the second year regardless of plant size. Both monocarpy and delayed reproduction allow a plant to make a large investment in reproduction (Klinkhamer and de Jong 1987). In a survey of life history and plant architecture, Schat et al. (1989) found that 56% of monocarpic perennials of the Dutch flora shared the following architectural traits: 1) a thick taproot; 2) a basal leaf rosette; 3) a robust erect leaf-bearing stem; and 4) either terminal or auxiliary inflorescences. Such traits are important for plants that bolt early in the spring and suggest an important role for floral display height in pollinator attraction.

Both species used in my experiment are able to continue photosynthesis through at least part of the winter, and such traits as a basal rosette and a thick taproot are key components for survival over the colder winter months. A basal rosette allows for a natural insulating layer of snow to reduce the negative freezing effect of colder temperatures experienced by taller plants. A taproot provides a vital connection to unfrozen water deeper underground to allow the plant to continue reduced photosynthesis throughout the winter months. Combined these traits support early bolting and flowering.

1.5 Size and reproduction in biennials and monocarpic perennials

The switch from vegetative rosette to reproductive stem elongation in monocarpic perennials is determined by plant size rather than age, such that an individual must reach a certain critical size before switching to reproduction (Lacey 1986). For biennials, the switch to reproduction occurs in the second season regardless of size. A monocarpic perennial life-history is advantageous when there is a more-than-proportional gain in fitness with size (Schat et al. 1989). Such a gain may come about from a more-than-proportional increase in reproductive output with plant size or if larger plants produce seeds of higher genetic quality (Stearns 1992). Such a disproportionate increase in reproductive output with size may occur from a disproportionate access to resources by larger plants. Since the plant architecture of the biennial life-history promotes seed production by repeated vertical units on a long stem, the cost per seed of producing increasing numbers of seeds decreases because much of the initial cost is in the production of the flowering stem and subsequent production of seed units require proportionally less total energy (Schat et al. 1989). This leads to an allometric relationship between increasing seed number and stem size. Therefore larger plants can allocate proportionately more energy to seed production, as the cost to a larger plant is less than a smaller one due to the initial cost of the stem production.

1.6 Study species

To investigate the role of plant height I chose two common mustard species: Garlic mustard (*Alliaria petiolata*) and Dame's rocket (*Hesperis matronalis*). The monocarpic nature of these two species provides an ideal system with which to study the fitness consequences of floral display height as they both experience a single reproductive episode thereby allowing lifetime maternal reproductive success to be accurately measured in terms of seed number and seed size. *A. petiolata* is an obligate biennial, known to be a highly selfing species exhibiting delayed self pollination (know as facultative xenogamous flowering) in which the small white flowers self pollinate after the second day of opening, ensuring complete pollination when pollinator visitation is low. *H. matronalis* is a monocarpic perennial that can flower in the first year but often delays bolting until its second year (Gleason and Cronquist 1991) and is known to be a predominant outcrosser (Mitchell and Ankeny 2001), with larger, showier, purple to white flowers, a more tube like corolla, and larger nectar reward than *A. petiolata*.

Both species grow in dense mono-specific stands. However, *A. petiolata* grows in larger, denser stands than does *H. matronalis* and, on average, is smaller than *H. matronalis*. These two species provide clear examples of two quite different points on the mating system spectrum and allow one to compare and contrast the effects and intensity of natural selection on floral display height and its consequences on fitness.

Both *H. matronalis* and *A. petiolata* often exist in high-density stands where intraspecific competition is high and where small differences in fitness can be magnified by such competition. Both *A. petiolata* and *H. matronalis* seeds germinate very early on in the spring, often producing hypocotyls under the snow, suggesting the importance of early germination to seedling establishment. It is likely that germination fraction and time-to-seed-germination are the most important traits in early plant fitness. Such traits may allow for early seedlings to become dominant in the highly competitive environment of dense monospecific stands (Schmitt and Ehrhardt 1990). Once established, such seedlings will generally out-compete later germinating seedlings for light and will likely be the ones to grow largest and tallest, and produce the highest floral displays. Thus, time-to-germination and percent germination may be good indicators of future plant success.

In this thesis, I explore the effect of floral display height on pollinator attraction and maternal fitness by first observing natural pollinator behaviour and then experimentally reducing the correlation between height and size by artificially varying plant stature while maintaining a normal plant size distribution. I do this in two closely related plant species with different mating systems but similar life histories. Secondly, I explore the effects of parental outcrossing or selfing on seed germination time and seedling growth traits for *Alliaria petiolata* to investigate the effects of inbreeding on maternal fitness. I ask the following questions: (*i*) do pollinators prefer taller floral displays, and if so does the increased visitation frequency lead to measurable differences in fitness? (*ii*) at what life-history stage are such fitness differences evident? (*iii*) does the importance of floral height display on plant fitness vary between mating systems?

CHAPTER 2

EFFECTS OF FLORAL DISPLAY HEIGHT ON POLLINATOR VISITATION AND REPRODUCTION IN *HESPERIS MATRONALIS* (BRASSICACEAE).

2.1 INTRODUCTION

For many plants, animal pollinators are essential to reproductive success. The frequency and efficiency of visiting pollinators often determines the reproductive output of a plant (Grant 1949, Kiester et al. 1984, Gramaldi 1999). To this end, flowering plants have evolved diverse mechanisms of co-opting pollination services to maximize fitness advantages (Feinsinger 1984, Stebbins 1984). The manipulation of pollinators by plants can take on many forms and is often linked to the traits used by pollinators to find flowers (Stebbins 1970, Faegri and van der Pijl 1979). Several studies have shown the importance of pollinator attractants such as flower size (Robertson and Macnair 1995), shape (Grant and Grant 1965, Inouye 1980), colour (Delph and Lively 1989, Melendez-Ackerman et al. 1997, Spaethe et al. 2001), nectar quantity and quality (Harder 1986, Wells et al. 1992), and flowering time (Schemske 1977, O'Neil 1999). However, few studies have looked at the pollinator attractiveness of floral display height (Pyke 1981, Larson and Larson 1990, Donnelly et al. 1998).

Insect biology suggests a role for floral display height. Many potential pollinators, especially bees, use sight as the main method of navigating to food sources. The maximum distance from which a bee can detect a food source is correlated to the size of the eye, with larger bees usually having larger eyes (Dafni and Kevan 1995). Taller floral displays project out above the surrounding vegetation, making them more conspicuous from greater distances, while shorter floral displays are hidden in the vegetative layer and are much less visible from afar. Flying in a straight horizontal line between floral displays is the optimal flight path when the vertical distance between flowers exceeds the horizontal (Levin and Kerster 1973). Thus a pollinator flies more efficiently by keeping on a horizontal trajectory between tall floral displays rather than flying up and down between short and tall flowers.

Optimal foraging theory (Schaffer and Schaffer 1979, Larson and Larson 1990) suggests that taller floral displays may attract more pollinators because pollinators could use height as a cue for efficient foraging. Since plant height is strongly correlated with plant mass, and plant height determines floral display height, pollinators may simply be using floral display height as a visual cue for detecting larger plants. This may be important to pollinators if larger plants produce more food rewards per flower cluster (i.e. more nectar or pollen) (Klinkhamer et al. 1991, Biernaskie and Elle 2005) or if larger plants produce food rewards of higher quality (i.e. higher sugar content of nectar). Because larger plants have greater access to both nutrients and light, it is likely that they may be able to produce more nectar or nectar of higher quality.

Other authors have reported on the effect of floral height on pollinator attraction. A survey of *Verbascum thapsus* revealed that pollinators visited plants that were significantly taller then their unvisited nearest neighbour and that seed set was significantly reduced when pollinators were excluded, despite the fact that this species experiences delayed selfing (Donnelly et at. 1998). Larson and Larson (1998) found in a population of *Spiranthes romanzoffiana* that bumblebees visited plants with the tallest spikes significantly more frequently then expected from random, and Pyke (1981) found that seed set increased with increasing floral display height in *Telopea speciosissima*.

However, floral display height and plant size are strongly correlated, so that a higher seed set or visitation frequency among plants with taller floral displays may simply reflect other advantages of being large plants. However, no one has yet experimentally and statistically separated the effects of height from size in pollinator attraction.

Hesperis matronalis offers an ideal system to study the effects of floral display height on plant fitness. As a monocarpic perennial, it grows initially as a rosette and only increases in height during the transition to flowering. Plant size is known to be a key factor in determining the switch from vegetative to reproductive modes, where rosette size is a better predictor of flowering than rosette age (Werner 1975, Gross 1981). This suggests that the selective advantage of delayed flowering is strongly linked to reproduction as a large plant (Schat et al. 1989). Delaying flowering to the second year is only advantageous if the reproductive output compensates for the risk of higher mortality in the vegetative stage (Hart 1977). A monocarpic perennial life history may therefore be advantageous when there is a more-than-proportional fitness gain with size (Schaffer and Gadgil 1975). Large plant size may confer a fitness advantage through higher rates of pollinator visitation since floral display height is strongly linked to plant size (Schat et al. 1989).

Antagonistic visitors such as herbivores, nectar robbers, phloem feeders, and seed predators may affect natural selection on floral characters (Aarsen 1995, Galen and Cuba 2001, Cariveau et al. 2004). The effect of attracting antagonists may be a reduction in the directional selection on floral traits by pollinators (Brody 1992, Cariveau et al. 2004) or a reduction in available resources for flower and seed production (Hamback 2001) particularly if pre-dispersal seed predators rely on the same cues that pollinators use to find potential food sources. Thus, tall floral displays, which may be good at attracting pollinators, may also attract unwanted pre-dispersal seed predators, leading to conflicting selection for floral traits (Cariveau et al. 2004).

In this study, I explored the fitness consequences of floral display height in the monocarpic perennial *Hesperis matronalis*. In field sites, I observed natural pollination and identified pollinators to morphospecies. Field-collected rosettes were transplanted into pots in a garden site. By raising the potted plants to different heights in an experimental array, the elevation of floral displays were manipulated independent of plant mass. Insect pollinators were observed in the experimental plant patch and pollinator visits to individual plants were recorded to assess the direct effects of floral display height on pollinator preference. I compared the effects of mass versus true plant height and experimental plant height on reproductive output and pollinator visitation frequency versus pre-dispersal seed predators on reproductive output. Specifically, I asked the following question; (*i*) does floral display height affect pollinator visitation independently of plant size and (*ii*) how do floral display height and plant size affect reproductive output? Because we observed evidence of pre-dispersal seed predation on the experimental plants, I also ask (*iii*) how does floral display height affect the presence of pre-dispersal seed predation? And (*iv*) how does plant size effect pre-dispersal seed predation?

2.2 MATERIALS AND METHODS

2.2.1 Study species

Hesperis matronalis (Brassicaceae) is a non-native monocarpic perennial in southern Ontario, Canada. After germination in early spring, it usually spends its first year as a rosette and then bolts in the following spring, producing a long thin stem 50 to 120 cm tall with smaller leaves and 1 to 7 inflorescences that produce many purple, pink, or white conspicuous flowers that are 1.2 cm in diameter. Flowering ends in late spring or early summer, at which point the long, slender fruits (siliques) ripen and disperse their seeds by late fall. The conspicuous flowers suggest this species is dependent on pollinators and it has been show to be highly outcrossing (Mitchell and Ankeny 2001). *H. matronalis* grows in open to semi shaded disturbed habitat, is often found in dense patches, and produces large groups of flowers at various heights. In southern Ontario, *H. matronalis* usually experiences a single reproductive episode in its second year; however, it may flower in its first year if conditions are favourable to allow the rosette to attain a critical minimum size.

2.2.2 Pollinator observations

Natural pollinator observations were carried out at two separate locations on properties of the Royal Botanical Gardens. Site 1 was a roadside plot on the west side of valley road between York road and Patterson road. Site 2 was an old-field site on the north side of Cootes drive between Olympic drive and Dundas street. At each site a plot of 6 m^2 (2 by 3 m) within a natural stand of *H. matronalis* was marked off and insect pollinators were observed. For each site, I recorded the height from the ground of visited flower clusters for as many as 4 stops on the pollinator's trajectories through the plot, after which pollinators were collected and identified to family and morphospecies. A floral display was recorded as having been visited when a pollinator landed on any flower in the cluster. Pollinators were observed at both sites for 1 hour between 11 am and 2 pm on 3 separate days during peak flowering in June of 2003 and 2004. Plants were then systematically pulled out of the plots and display height measured from the base of each stalk to each flower cluster. From this, I calculated mean floral display height for all plants and for those visited by pollinators. Plant density was estimated by dividing the number of plants per plot by the area of each plot. Data were pooled from the two sites since there were no significant differences in pollinator assemblage, floral display height of each site, nor pollinator visited floral display height between sites. I did not measure the number of open flowers in each flower cluster, as this changed over the time period that pollinator visits were observed.

2.2.3 Height manipulation experiment

During May of 2004, I collected second year rosettes of *H. matronalis* from the Royal Botanical Gardens (RBG) property along the roadside off Valley Rd. between York drive and Rock Chapel road in Hamilton, Ontario, Canada. I collected 140 plants and transplanted them into styrofoam cups (10 cm diameter and 14 cm tall, hereafter referred to as pots). Plants were transferred to the RBG boathouse garden, near the north shore of Cootes Paradise, where, after 4 days of acclimation, selected plants were labelled and placed into a 10 x 10 block in an area of the boat house garden that was semi-shaded and 1.5 m from the edge of natural old field. Pots were placed as close together as possible such that plants were approximately 10 cm apart (100 plants m^{-2}), with half of them assigned to an elevated treatment and half to a non-elevated treatment. Plants were randomly assigned to each treatment and treatments were randomly distributed within the experimental plot.

Pots of non-elevated treatment plants were positioned directly on the ground. Pots of elevated treatment plants were raised 20 cm by placing them atop over-turned plastic pots. All plants were staked down using 1 m long bamboo stakes to stop them from falling over. A border row of plants was later discarded from the data set to remove edge effects.

Plants began bolting soon after placement into their treatment. On May 21, 2004, plant height was recorded for all treatment plants. Plant height was defined as the true height of the plant irrespective of elevation treatment, i.e., the measurement of the central flowering stalk from its base to apex. By May 27, 2004 all but 6 plants were blooming. Treatment plants were visited every few days and watered as needed. For 6 days between May 27 and June 9, I observed pollinators visiting flowers in the plot for a one-hour period between 11 and 3 pm, and recorded pollinator types and plants visited. A pollinator was scored as a visitor to a plant when it landed on a flower cluster, and pollinator visitation frequency was calculated as the total number of pollinator visitors observed for each plant in the plot.

Plant height was again measured on July 8, 2004, at which point all plants had green ripening fruit and were no longer flowering. At this point, experimental plants were removed from their treatments and were spaced out on the ground to remove any treatment effect on further growth and ripening of fruit. Plants were harvested on August 5, 2004 by cutting the plants at the base and placing them into paper bags for drying. After air drying in the lab for several weeks, data were collected on total above ground plant mass, vegetative mass, total reproductive mass, seed mass, husk mass, number of fruit and number of seeds.

I calculated average seed mass as total seed mass divided by total number of seeds and average number of seeds per fruit as total number of seeds divided by the number of fruit.

Damage to fruit and seeds was evident among some plants. I measured the number of damaged fruit per plant and the number of damaged seeds per plant. Damaged fruit were any that had small, blackened puncture holes (indicating insect feeding), while physically altered seeds or frass left in the footprint of a seed inside each fruit were counted as damaged seeds. I calculated relative seed damage as the number of damaged seeds divided by the total number of non-damaged seeds per plant (Log transformed).

2.2.4 Statistical analysis

All analyses in this study were conducted with JMP version 4.0.4 (SAS Institute 1999) for Macintosh. Relative seed damage and relative fruit damage were log transformed to meet the requirements of normal distribution of the residuals. A transformation of all other data was not necessary as the residuals were normally distributed. Type III sums of squares were used for all analyses of variance and covariance. Differences between elevation treatments for plant fitness traits were contrasted using one-way analysis of variance (ANOVA). The difference in pollinator visitation between treatments was compared using a Wilcoxon nonparametric test because of the non-continuous nature of pollinator data. I used two statistical methodologies to separate the effects of plant height from plant size. In the first, I used analysis of covariance (ANCOVA) to assess which measures of pollinator visitation and reproduction (pollinator visitation frequency, mean seed mass, number of damaged fruit, fruit per gram vegetative mass, and log transformed relative seed damage) were affected by the elevation treatment with vegetative mass included as a covariate. In the second, I used path analysis, which combines standardized regression coefficients and an *a priori* causal hypothesis to determine how strongly traits affect one another (Li 1986). Plant size and experimental plant height (true height plus elevation treatment) were used as independent variables, and all reproductive traits as dependent variables. I also modelled causal dependencies among reproductive traits.

2.3 RESULTS

The natural field sites had an overall plant density of 36.75 ± 1.95 (mean ± SE) plants m^{-2}, with plants patchy within plots. Mean floral display height was 84.24 ± 0.68 cm and mean height of insect visited floral displays was 83.19 ± 1.59 cm. There was no significant difference between the mean population height of all floral displays and the mean height of insect visited floral displays (one-way ANOVA $F = 0.459$, $P = 0.5$, $df = 1$). The pollinator assemblage in the two field sites was dominated by four families of bees, and one family of fly (Table 2.1). One species of the family Helictidae was observed nectar robbing, by chewing into the base of flowers. Some long horned beetle species (Cerambycidae) were observed on *H. matronalis* flowers outside the site but only one was collected from the experimental plants. Pollinators at the experimental plot were approximately in the same ratio of bees to flies (roughly 3:1) that was observed in the natural sites; however, the overall diversity was lower at the experimental site (Table 2.1).

In the experimental plot, visitation frequency was significantly and positively affected by plant height, the number of fruit, and above ground vegetative mass. The number of flower stalks had no effect on pollinator visitation frequency (Table 2.2). Pollinator visitation increased with increasing plant height (Figure 2.1) and elevated treatment plants were visited by pollinators significantly more frequently than were non-elevated treatment plants (Wilcoxon nonparametric test, $\chi^2 = 4.6872$, $P = 0.0304$, $df = 1$) (Figure 2.2).

The height manipulation did not alter growth patterns in *Hesperis matronalis*. One-way ANOVA revealed that the elevation treatment had no significant effect on plant height ($F = 0.055$, $P = 0.820$ $df = 1$), vegetative mass ($F = 1.261$, $P = 0.265$, $df = 1$) or the number of flowering stalks ($F = 2.279$, $P = 0.135$, $df = 1$).

In both treatments, the number of seeds produced was strongly and positively correlated to plant vegetative mass (Table 2.3, Figure 2.3). Total seed mass and the number of fruit were not significantly affected by the elevation treatment, while number of seeds, and seeds per fruit increased significantly in the elevated treatments (Table 2.3, Figure 2.4). Mean seed mass was significantly lower among elevated plants (Figure 2.5). Surprisingly, the effect of height on mean seed mass depended on the elevation treatment: taller plants had smaller seeds among elevated treatment plants but mean seed mass was uncorrelated with height in non-elevated treatment plants (Table 2.3, Figure 2.6). There was a significant treatment × vegetative mass interaction for total seed mass (Table 2.3). Larger plants in the elevated treatment had lower total seed mass than larger plants in the non-elevated treatment, whereas smaller plants in the elevated treatment produced higher total seed mass than their counterparts in the non-elevated treatment, suggesting a cost of elevation (Figure 2.7).

Vegetative mass had a significant and positive effect on plant height, the number of flowering stalks, total seed mass, number of seeds and the number of fruit (Table 2.3). However, vegetative mass had no significant effect on average seed mass or the number of seeds per fruit (Table 2.3).

The size of the path coefficient measures the relative importance of the independent predictors, which are experimental height (true plant height plus elevation treatment) and plant vegetative mass. Path analysis indicated that vegetative mass was more important in both fruit and seed production, while height and vegetative mass were equally important in pollinator attraction (Table 2.4). When using a more complex model, vegetative mass had no significant direct effect on seed number. Instead vegetative mass increased seed production indirectly by significantly increasing the number of fruit, while plant height significantly and positively affected seed number directly, but had no significant effect on number of fruit (Figure 2.8). Mean seed mass strongly decreased by increased height, but was unaffected by plant size (Table 2.4)

Analysis of covariance indicated no significant effect of elevation treatment (F = 0.267, P = 0.61), or plant vegetative mass (F = 2.67, P = 0.11) on fruit damage. Log relative seed damage was not significantly affected by elevation treatment (Table 2.5). However, there was a significant effect of vegetative mass and a significant interaction between vegetative mass and elevation treatment on log relative seed damage (Table 2.5). Log relative seed damage decreased as plant mass increased in the non-elevated treatment, yet log relative seed damage was not related to plant mass in the elevated treatment (Figure 2.9).

2.4 DISCUSSION

Determining the role of floral display height and plant size in reproduction is difficult since these two traits are strongly correlated. But, by manipulating height and, so, reducing the correlation between height and size in an experimental population, I was able to measure the effects of each trait separately. As predicted in this experimental population, taller plants attracted more pollinators and produced more seeds. However, surprisingly, taller plants produced smaller seeds.

The height effects on pollinator attraction in the experimental population contrasted with field observations, where floral display height seemed to have no effect on pollinator visits. This apparent contradiction may be due to a lower plant density in the field sites as compared to the experimental array (37 plant m^{-2} versus 100 plants m^{-2}). Optimal foraging theory dictates that a pollinator should fly a horizontal line between flowers only when the vertical distance between flowers exceeds the horizontal (Levin and Kerster 1973). Thus, pollinators visiting plants in lower density stands may forage optimally by visiting flowers at various heights. It may also be the case that the lower density of the field sites simply made the flowers of shorter plants more visible, whereas, in the high density experimental stands, the shorter floral displays were more hidden in the denser vegetation.

Analysis of covariance showed that both larger plants and taller plants attracted more pollinators and produced more seeds. Path analysis demonstrated that plant mass increased seed numbers indirectly by producing more fruit while experimental plant height increased seed numbers directly by producing more seed per fruit, presumably the outcome of more pollinator visits to taller plants. However plant size, the number of flower stalks, and the number of fruit also positively affected pollinator visitations suggesting that floral display height may be one in a complex set of cues used by pollinators in detecting food sources. Similar results have been reported for other monocarpic perennials. For example, in *Erysimum mediohispanicum*, pollinators preferred taller floral displays overall but also greater flower numbers, stems, and shorter corollas. And more frequently visited plants produced significantly more seeds (Gomez 2003).

If fitness is measured as seed number, then elevated plants are clearly more fit than non-elevated plants. Offspring quality, however, potentially differs between treatments, since non-elevated plants had larger seeds, and seed size is usually considered to increase offspring fitness (Gross 1984, Rees 1995, Weiner et al. 1997, Jakobsson and Eriksson 2000). However, in a review of germination studies, Baskin and Baskin (1998) found that the effect of seed size on germination time and germination fraction varied with the species studied and that it could be negatively or positively correlated or have no effect at all.

Genetic quality may also differ between treatments, since elevated plants more likely received more varied pollen and more non-self pollen as visiting pollinators bring pollen from many plant of varying fitness. A greater diversity of deposited pollen may lead to higher seed quality because this allows for greater mate choice. For example, Mitchell and Marshall (1998) demonstrated in the related species *Lesquerella fendleri* that non-random mating takes place post-pollination. They showed that, when a mixture of pollen with known parentage was added to female flowers, some paternal donors sired more than 70% of all seeds. Temme (1986) argues that the maternal investment in offspring should vary with the genetic quality of the zygote, leading to higher overall fitness for the maternal plant. Thus, detectable genetic variation should affect the optimal pattern of maternal allocation. Zygotes of lesser genetic quality will need more maternal provisioning than will ones of higher genetic quality to have the same chances of becoming established seedlings. Thus, maternal investment (seed size) compensates for lower genetic quality (Temme 1986). In this way, the maternal plant may spread its resources in the most optimal way to ensure maximum fitness advantage to the next generation.

In *H. matronalis*, larger plants did not produce bigger seeds, suggesting that increasing seed number rather than seed size provides for optimal parental fitness. This finding is consistent with the terminal stream limitation model (Sakai and Harada 2005), which proposes that assessing parental provisioning efficiency of trade-offs between seed number and seed size is a non-linear relationship such that total offspring mass increases with increasing seed number but decreases with increasing seed size because loss of resources via maintenance respiration decreases with offspring number but increases with offspring size. This model suggests that, in terms of resource use efficiency, increasing seed number rather than seed size is optimal.

The results showed that a clear seed size/number trade off occurred in experimental plants of *H. matronalis*. Because unused resources cannot be allocated to future growth or reproduction in monocarpic perennials, and since reproductive resources are limited, reproductive allocation must be optimised between the number of seeds produced and the size of each seed (Smith and Fretwell 1974, Lalonde and Roitberg 1989, Wolfe 1995). Such a seed size/number trade-off may have come about through pollen limitation or early inbreeding depression, and it is likely that both play a role in *H. matronalis* life history; however, the present study cannot distinguish between the two.

Total seed mass increased with plant vegetative mass in both treatments, yet the interaction effect indicates a significant cost to elongation among elevated plants. Being too tall appeared to have a significant cost on reproduction. This may be because, as elevated plants grow significantly above their neighbours, they lose the structural support offered by neighbours, making them susceptible to environmental stressors such as increased wind and desiccation, both of which can have negative effects on reproductive output (Bertness and Callaway 1994, Harley and Bertness 1996).

Other monocarpic perennials have a strong reliance on size and, consequently, height for successful pollination and maximum fruit set (Sletvold 2002, Gomez 2003). Large individuals of monocarpic species often emphasize female function (De Jong et al. 1989); consequently, size is more important for seed production than for pollen removal. This appears to be the case in *H. matronalis*, where elevated treatment plants produced significantly more seeds per fruit than did non-elevated treatment plants, suggesting height-dependent sex allocation. Similar results have been found in the facultative biennial *Digitalis purpurea*, where seed number increases proportionally with plant size but total seed mass increased disproportionally with size (Sletvold 2002).

Seed damage varied in part with plant mass and elevation. Among elevated plants, relative seed damage was constant with increasing plant mass. But, since relative seed damage decreased with increasing plant mass among non-elevated plants, both predispersal seed predation and pollinator preference appear to converge in selecting for increased plant size.

Table 2.1. Summary of insect pollinator diversity for two natural stands of *Hesperis matronalis* and number of pollinators in the experimental array (order only).

	Site 1		Site 2		Exp. plot
Families	Number of Morpho-species	Number of individuals	Number of Morpho-species	Number of individuals	Number of individuals
Bees – total	15	67	13	55	69
Andrenidae	7	33	6	24	
Anthophoridae	1	3	1	2	
Helictidae	6	25	5	19	
Apidae	1	6	1	10	
Butterflies					
Papilionidae	1	2	1	2	0
Flies					
Syrphidae	4	18	3	17	21
Beetles					
Cerambycidae	0	0	1	1	0
Total	20	87	18	75	90

Table 2.2. Analysis of covariance for factors affecting pollinator visitation in experimental plants of *Hesperis matronalis*.

Source	*df*	Sums of Squares	*F*-ratios	Prob > *F*
Elevation treatment	1	6.959	19.550	0.0001
No. of fruit	1	1.581	4.442	0.0385
Vegetative mass (g)	1	2.230	6.265	0.0146
Plant height (cm)	1	2.508	7.047	0.0097
Flower stalks	1	1.003	2.819	0.0974
Elevation treatment × vegetative mass (g)	1	1.546	4.345	0.0406
Error	73			

Table 2.3. Analysis of covariance for fitness traits in experimental plants of *Hesperis matronalis*. Where elevation treatment × vegetative mass was not significant, it was omitted from the model.

Source	*df*	*F*-ratios				
		Total seed mass	Number of seeds	Average seed mass	Number of fruits	Number of seeds/fruit
Elevation treatment	1	0.04	5.61*	9.52**	0.91	4.78*
Vegetative mass	1	82.7***	60.4***	0.75	106.8***	0.22
Elevation treatment × vegetative mass	1	4.37*				
Error	76					

$*P < 0.05$, $**P < 0.01$, $***P < 0.001$

Table 2.4. Magnitude of direct effect (path coefficients) of standardized vegetative mass and experimental plant height (actual plant height plus treatment height) on standardized reproductive traits in *Hesperis matronalis*. The correlation between experimental height and vegetative mass was $r = 0.54$, $P < 0.0001$. The correlation between true plant height and vegetative mass was $r = 0.79$, $P < 0.0001$.

Path coefficient Vegetative mass	Path coefficient Exp. height	Reproductive trait
0.40***	0.51***	Pollinator visits
0.51***	0.22**	Number of seeds
0.21	-0.40***	Seed mass
0.77***	0.02	Number of fruit

*$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 2.5. Analysis of covariance of the effects of vegetative mass and height manipulation treatment on log transformed seed damage in *Hesperis matronalis*. Log transformed relative seed damage was calculated as the log of total damaged seeds per plants divided by total seeds per plant.

Source	*df*	Sums of Squares	Log relative seed damage	*P* value
Elevation treatment	1	0.8071	0.6800	0.4133
Vegetative mass	1	4.9858	4.2010	0.0454
Elevation treatment × vegetative mass	1	5.7298	4.8279	0.0324
Error	57			

Figure 2.1. A scatter plot showing pollinator visitation as a function of plant height for experimental plants of *Hesperis matronalis*. Pollinator visitation frequency increases with plant height in both treatments. Linear regression for elevated plants: $y = 0.072x - 3.83$, $r^2 = 0.81$, $n = 41$. Linear regression for non-elevated plants: $y = 0.041x - 2.19$, $r^2 = 0.64$, $n = 39$. Regression values for elevated ($F = 75.0$) and non-elevated ($F = 25.7$) plants were both significant at $P < 0.0001$. Elevated treatment (dashed line) had a significantly higher mean visitation frequency than non-elevated treatment (Wilcoxon nonparametric test, $X^2 = 4.86$, $P < 0.027$, $df = 1$). Analysis of covariance revealed a significant interaction effect indicating that height manipulation treatment had a significant effect on pollinator visitation frequency ($F = 7.33$, $P = 0.0083$, $df = 1$). Y-axis is shifted slightly between treatments for clarity.

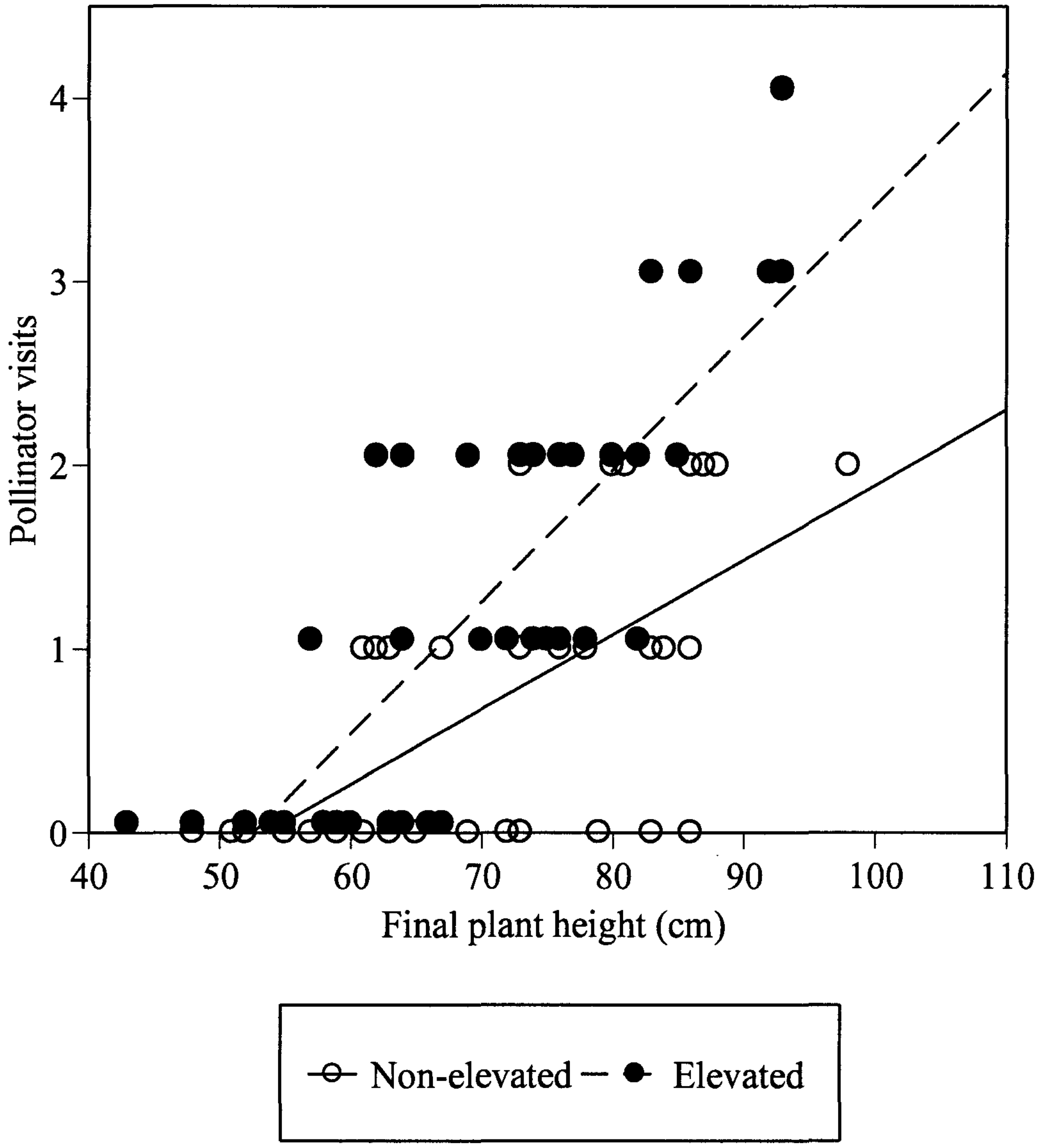

Pollinator visits
0
1
2
3
4
40
50
60
70
80
90
100
110
Final plant height (cm)
Non-elevated
Elevated

Figure 2.2. Mean frequency of pollinator visitation between non-elevated and elevated treatment plants of *Hesperis matronalis* in the experimental plot with bars indicating 1 SE. Means are significantly different (Wilcoxon nonparametric test, $\chi^2 = 4.687$, $P = 0.03$, $df=1$). $N = 77$.

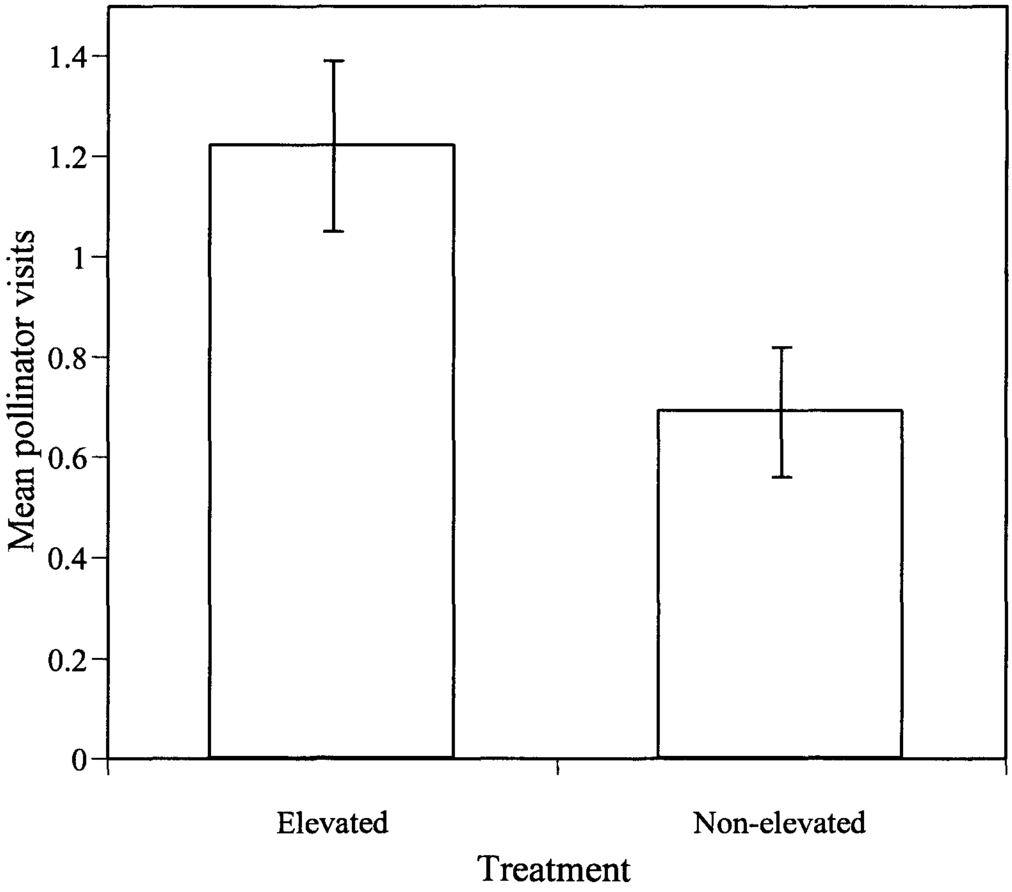

1.4
1.2
1
0.8
0.6
0.4
0.2
0
Mean pollinator visits
Elevated
Non-elevated
Treatment

Figure 2.3. The relationship between plant vegetative mass and numbers of seeds produced for elevated and non-elevated treatment plants of *Hesperis matronalis.* The number of seeds increases with vegetative mass in both treatments. Linear regression for elevated plants: $y = 112.8x + 82.5$, $r^2 = 0.71$, $n = 41$. Linear regression for non-elevated plants: $y = 128.6x + 28.7$, $r^2 = 0.72$, $n = 39$. All regression values (elevated, $F = 39.0$; non-elevated, $F = 39.5$) were significant at $P < 0.0001$. Analysis of covariance indicates a significant effect of elevation treatment (see table 2.3), but no significant difference between slopes ($F = 0.31$, $P = 0.58$, $df = 1$).

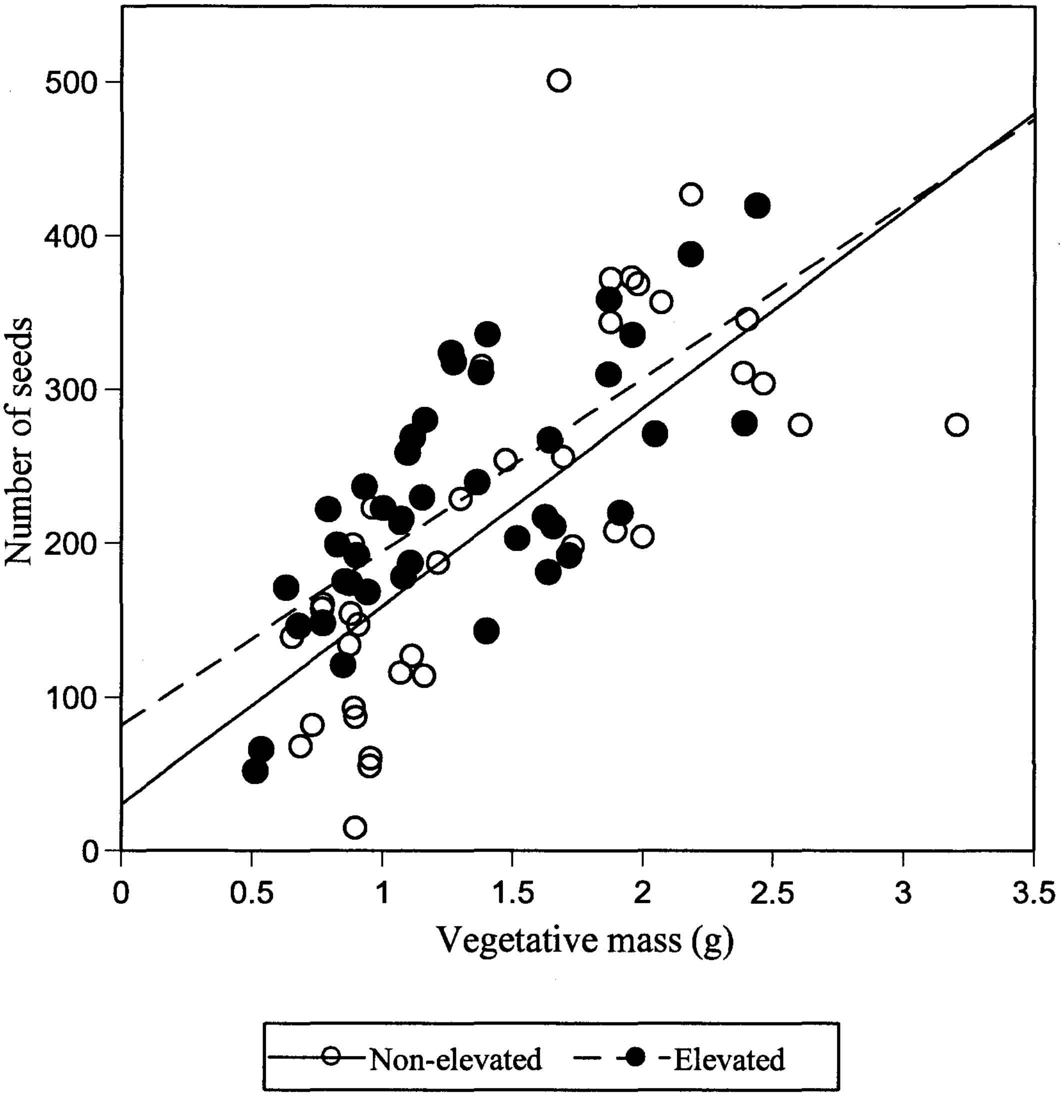

Number of seeds
0
100
200
300
400
500
0
0.5
1
1.5
2
2.5
3
3.5
Vegetative mass (g)
Non-elevated
Elevated

Figure 2.4. A comparison of the mean number of seeds produced per fruit for elevated (n = 41) and non-elevated (n = 39) treatment plants of *Hesperis matronalis*. Means are significantly different (one-way ANOVA: F=4.65, P=0.03, df= 1). Bars indicate 1 SE.

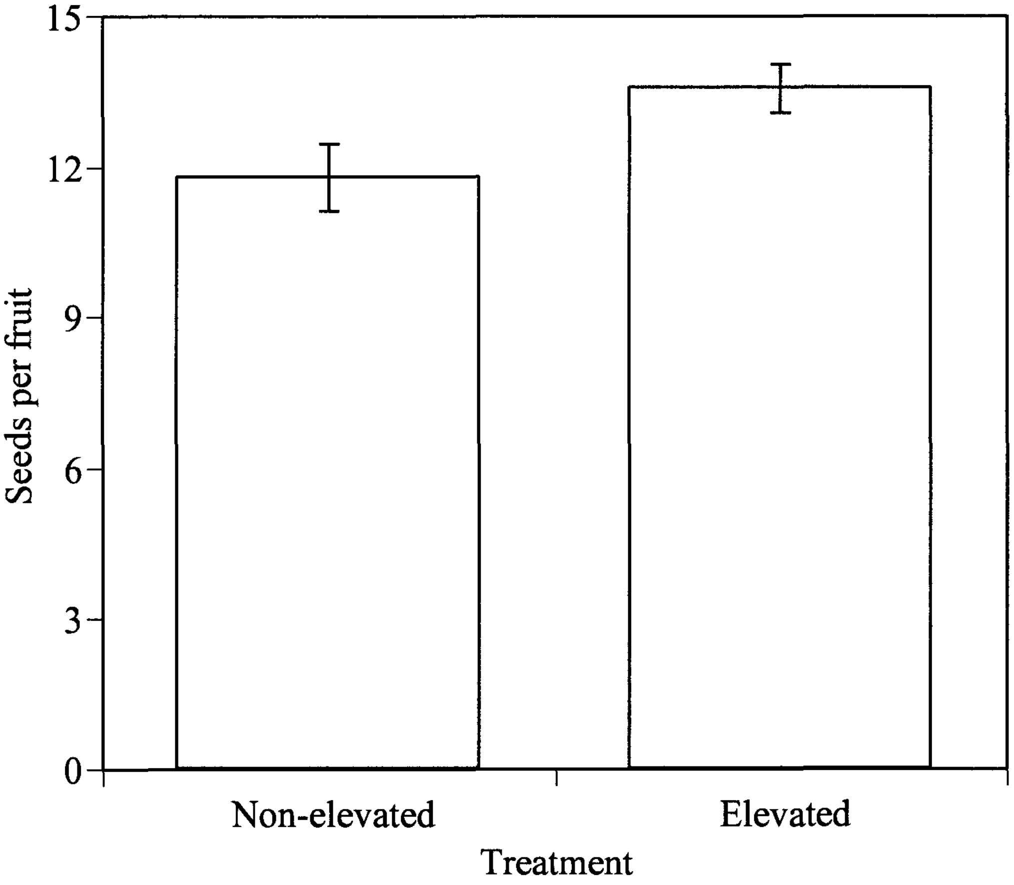

15
12
9
6
3
0
Seeds per fruit
Non-elevated
Elevated
Treatment

Figure 2.5. A comparison of mean seed mass for non-elevated ($n = 41$) and elevated ($n = 39$) treatment plants of *Hesperis matronalis*. Means are significantly different based on a one-way ANOVA ($F = 8.66$, $P = 0.0043$). Bars indicate 1 SE.

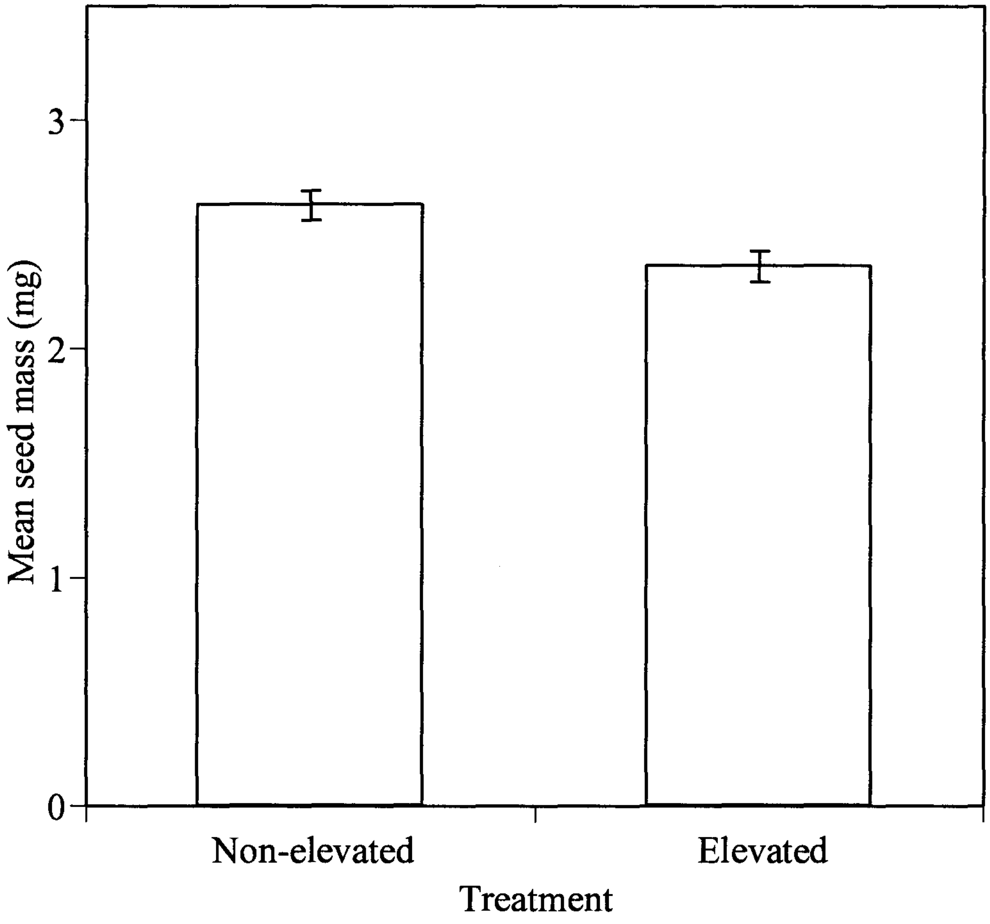

Mean seed mass (mg)
0
1
2
3
Non-elevated
Elevated
Treatment

Figure 2.6. The relationship between the mean seed mass and final plant height for elevated and non-elevated treatment plants of *Hesperis matronalis*. Linear regression for non-elevated plants $y = 2.49\text{x}10^{-3}x + 2.45$, $r^2 = 0.08$, $n = 39$. Linear regression for elevated plants $y = 1.44\text{x}10^{-2}x + 3.36$, $r^2 = -0.40$, $n = 39$. Plant height had no significant effect on mean seed mass for non-elevated treatment plants ($F = 0.22$, $P = 0.64$, $df = 1$), but a significant negative effect on elevated treatment plants ($F = 7.58$, $P = 0.89 \times 10^{-2}$, $df = 1$). Analysis of covariance revealed a significant effect of elevation treatment by plant height on mean seed mass ($F = 577$, $P = 0.019$, $df = 1$).

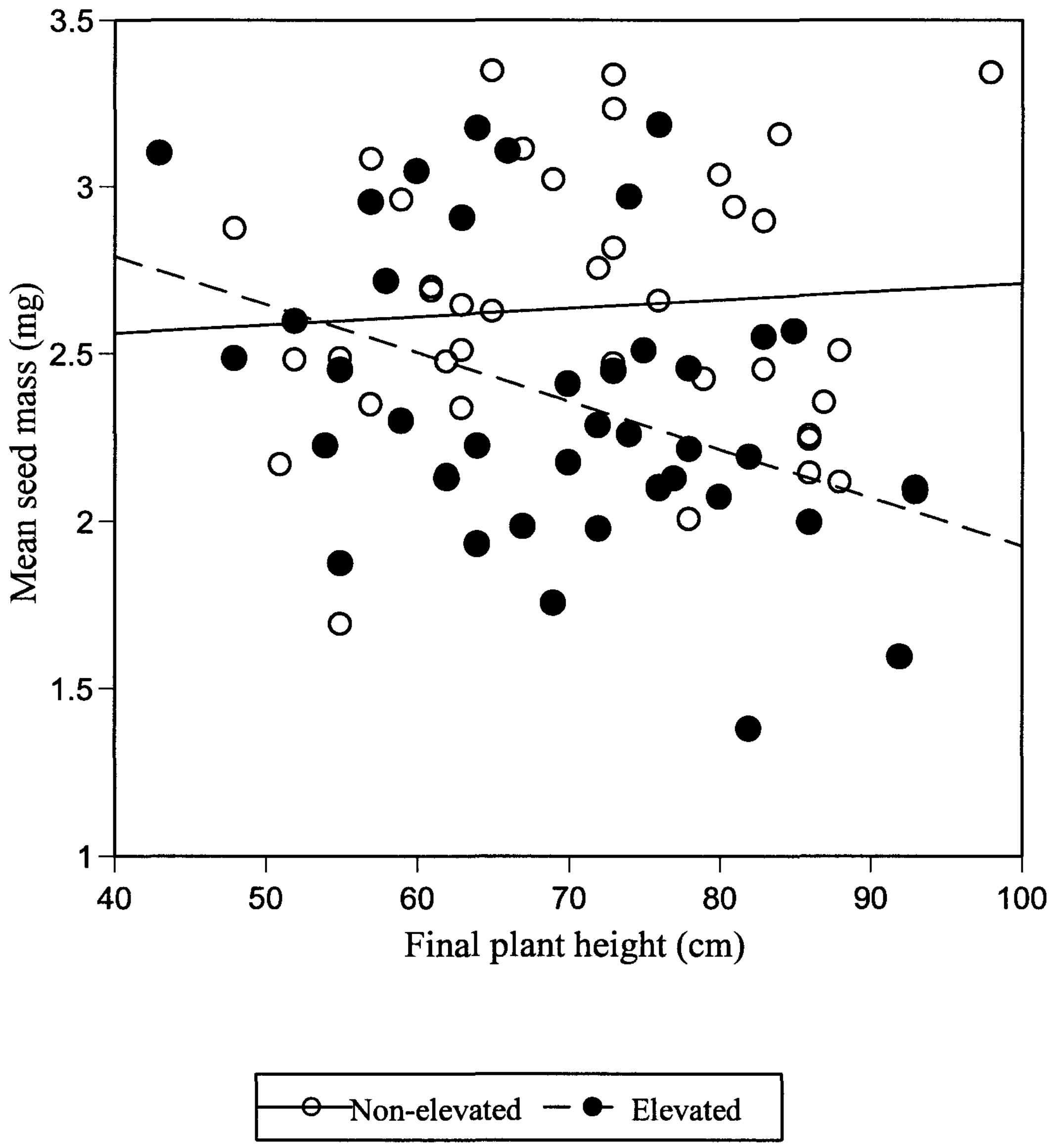

3.5
3
2.5
2
1.5
1
40
50
60
70
80
90
100
Mean seed mass (mg)
Final plant height (cm)
Non-elevated
Elevated

Figure 2.7. The relationship between total seed mass and vegetative mass between treatment plants of *Hesperis matronalis*. Linear regression for non-elevated plants: n = 39, y =0.35x + 0.047, $r^2 = 0.80$, $F = 66.3$, $P < 0.0001$. Linear regression for elevated plants, $n = 41$, y = 0.22x + 0.24, $r^2 = 0.63$, $F = 25.09$, $P < 0.0001$. Analysis of covariance indicated an elevation treatment × vegetative mass interaction on total seed mass (see Table 2.3).

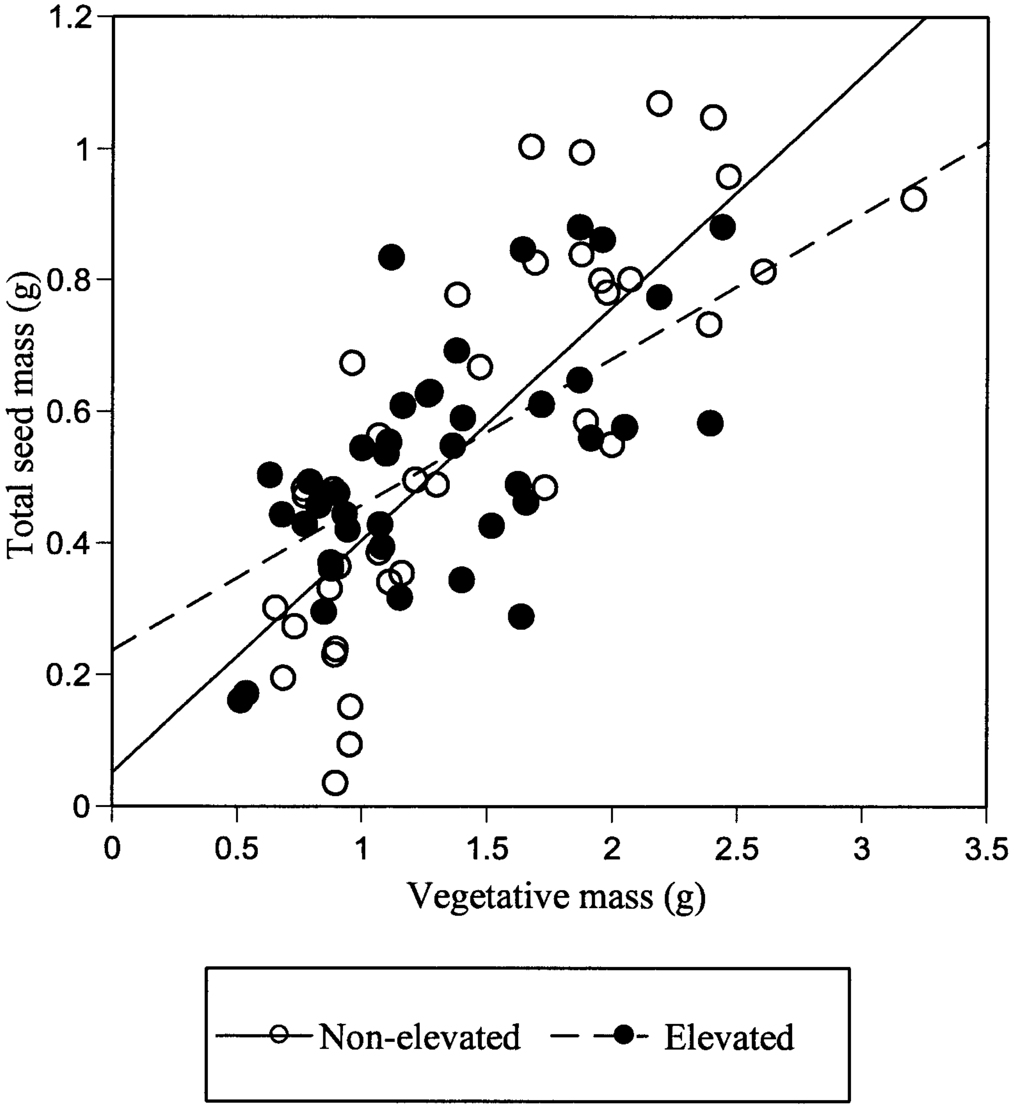

1.2
1
0.8
0.6
0.4
0.2
0
Total seed mass (g)
0
0.5
1
1.5
2
2.5
3
3.5
Vegetative mass (g)
Non-elevated
Elevated

Figure 2.8. A path diagram for the effects of vegetative mass and experimental plant height (height of actual plant + treatment height) on the number of fruit and number of seeds in experimental plants of *Hesperis matronalis*. The correlation between actual plant height and mass was $r = 0.79$. Path coefficients with significant values are marked as follows: *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Vegetative mass had a significant indirect effect on seed numbers via number of fruit, while experimental height has a direct and significant effect on seed numbers presumably from increased pollinator visits (see Table 2.4).

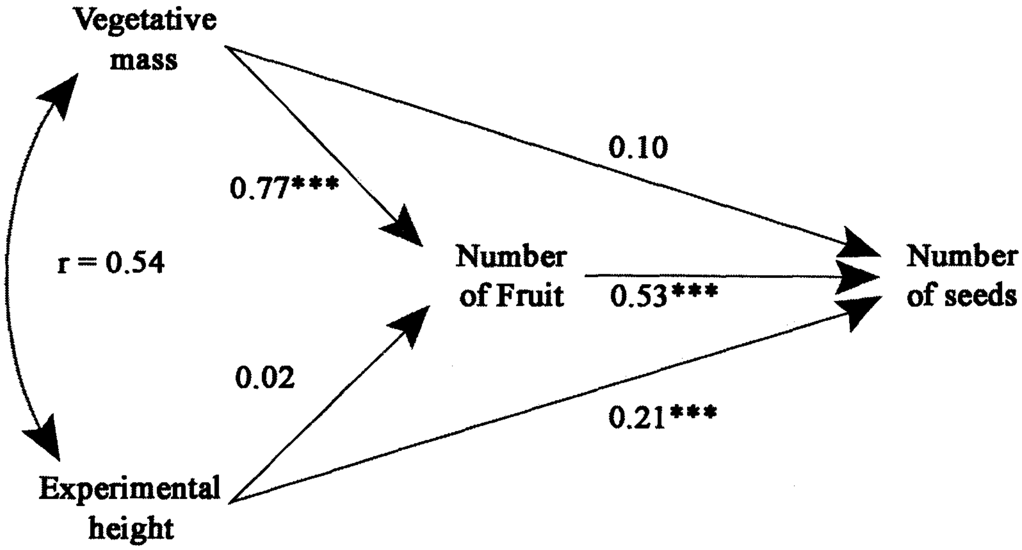
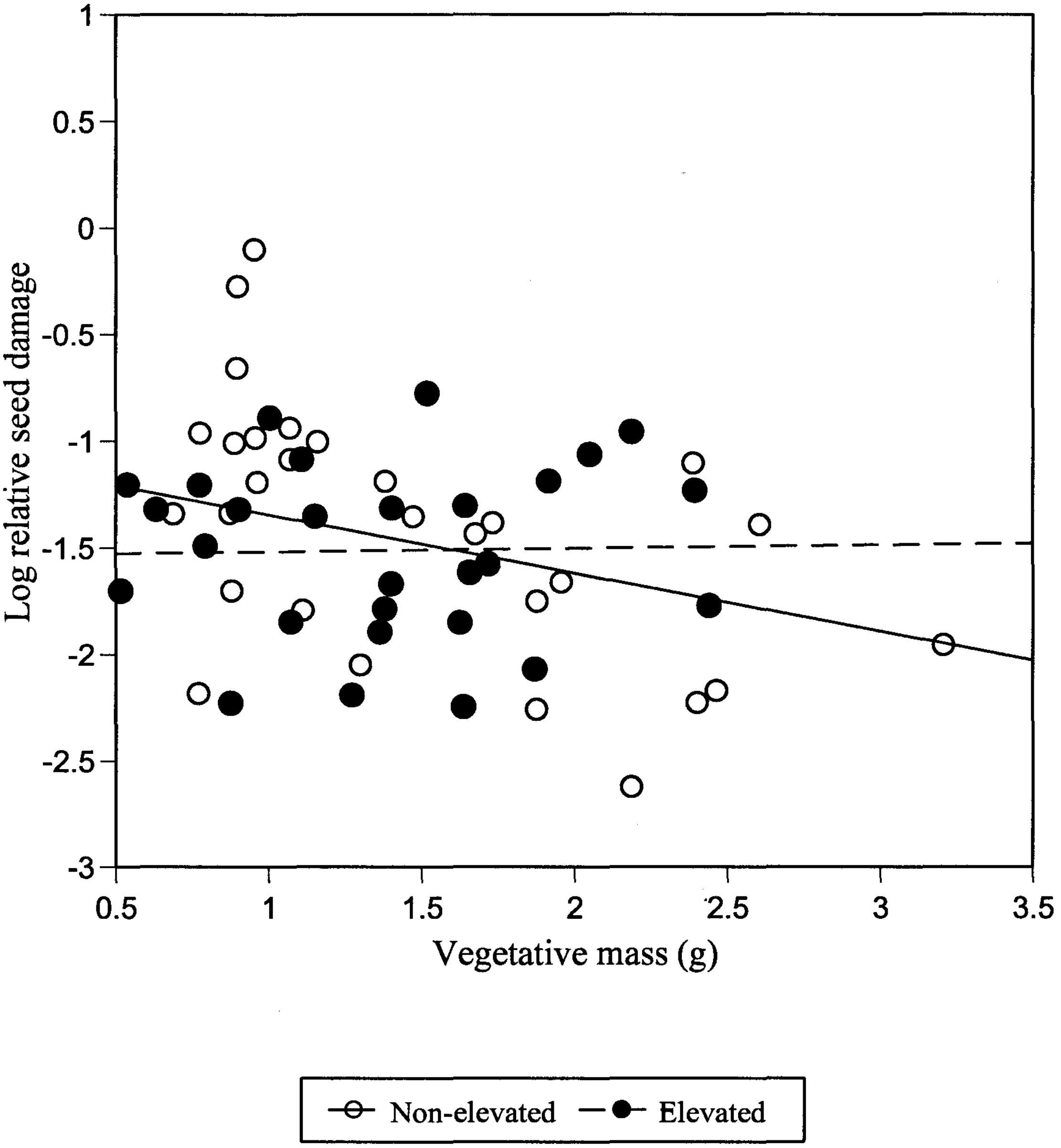

Vegetative mass
r = 0.54
0.77***
0.02
0.10
Number of Fruit
0.53***
0.21***
Number of seeds
Experimental height

Figure 2.9. Log transformed relative seed damage as a function of vegetative mass for non-elevated and elevated treatment plant of *Hesperis matronalis.* Non-elevated plants, $n = 29$, $y = -0.46x - 0.75$, $r^2 = -0.52$. Elevated plants, $n = 28$, $y = -0.016x + 1.53$, $r^2 = -0.02$. The regression was significant for non-elevated treatment only (non-elevated, $F = 9.943$, $P = 0.004$; elevated, $F = 0.011$, $P = 0.92$). ANCOVA revealed a significant interaction effect indicating that non-elevated plants experience less relative seed damage as vegetative mass increases ($F = 4.828$, $P = 0.032$).


Log relative seed damage
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
0.5
1
1.5
2
2.5
3
3.5
Vegetative mass (g)
Non-elevated
Elevated

CHAPTER 3

EFFECTS OF FLORAL DISPLAY HEIGHT, MATERNAL SIZE, AND POLLINATION ON REPRODUCTION IN THE PREDOMINANT SELF-POLLINATOR *ALLIARIA PETIOLATA* (BRASSICACEAE).

3.1 INTRODUCTION

For many plants, height increases in response to population density (Schmitt and Wulff 1993). Such density-dependent elongation has been shown to increase plant fitness via increased carbon acquisition in environments with limited light resources (Dudley and Schmitt 1996). However, in monocarpic perennials, stem elongation occurs regardless of plant density (Cavers et al. 1979) as part of the transition to reproduction, suggesting that elongation may serve another function. In chapter 2, I demonstrated that in *Hesperis matronalis*, a monocarpic perennial, taller plants produce more yet smaller seeds than do shorter plants and that plant height is a key factor in attracting pollinators independent of plant size. *Alliaria petiolata* is closely related to *H. matronalis*. Both share a common rosette-to-bolting morphology but differ in their mating strategies. *H. matronalis* is known as a predominant outcrosser (Mitchell and Ankeny 2001), while *A. petiolata* is know to be highly self-pollinating by a mechanism known as facultative xenogamous flowering, where self pollination occurs only after the potential for outcrossing has passed (Cruden et al. 1996). These differences in mating systems suggest that height is less likely to affect plant fitness through pollinator attraction in *A. petiolata* than in *H. matronalis.*

In a highly selfing species, pollinator attraction will only be important if there is inbreeding depression. Predominant self-pollinating species such as *A. petiolata* will tend to purge highly deleterious alleles, since homozygosity for deleterious recessive alleles would expose them to natural selection (Lande and Schemske 1985). However, if deleterious recessive alleles have only mildly deleterious effects or a low dominance coefficient, natural selection will not remove them entirely, and, so, inbreeding depression will persist in even a highly selfing population (Charlesworth et al. 1990b). Purging by inbreeding has also been shown not to act uniformly across life stages, and the degree of selfing or outcrossing within a species affects the magnitude and timing of inbreeding depression (Husband and Schemske 1996). Husband and Schemske (1996) examined 54 species of angiosperms and found that predominantly selfing species exhibited lower inbreeding depression rates than did outcrossed species, and they experience it later in their life stages; whereas predominantly outcrossing species experience inbreeding depression throughout, but especially in early stages such as seed production.

In high-density stands, minute genetic advantages in outcrossed seeds may be magnified by competition, a phenomenon known as dominance and suppression (Schmitt et al. 1986). Frequently, *A. petiolata* populations are large and grow in dense stands in which low seed dispersion results in a dense seed bank (Anderson et al. 1996). Because of the cyclical nature of this biennial species, individual stands tend to be monospecific and of uniform age so that seedlings are in intense competition with other seedlings and not second year rosettes. Intraspecific competition for light may frequently cause asymmetrical size distribution in plant populations with few large plants and many small plants (Weiner 1986). This provides the conditions in which dominance and suppression could result in an advantage for outcrossed individuals within this predominantly self-pollinating species.

A. petiolata provides an ideal system to investigate the effect of floral display height on fitness in a highly selfing species. The delayed selfing mechanism promotes outcrossing when pollinators are frequent while assuring a complete seed set when pollinators are rare (Cavers et al 1979). Thus, if pollinators are common, and pollinators prefer taller floral displays as has been shown for *H. matronalis* (Chapter 2), then taller plants should produce relatively more outcrossed seeds than do shorter plants. Also, because this species produces a single erect stem and most flowering occurs at the apex of the stem, the effect of floral display height on pollinator visitation should be readily observable. This hardy species often grows in high-density stands in S. Ontario that should bring about strong selection for any mechanism that may impart a genetic advantage in a highly competitive environment. Finally, the single reproductive episode in its second year allows for a complete assessment of lifetime fitness.

In the present study, I explore the fitness consequences of floral display height. I measure the role of floral display height on pollinator visitation frequency in a natural population. In the experimental array, I investigated the role of height on seed set and seed size. In a subsequent study, I measured the effects of artificial selfing and outcrossing on plant fecundity. And last, I measured the effects of maternal plant size, pollen source, and density in the progeny resulting from selfed and outcrossed plants. I ask the following questions: (*i*) do taller floral displays attract more pollinators? (*ii*) Do taller plants produce more seeds? (*iii*) Is there any inbreeding depression, and if so, at what stage? (i*v*) Does competition affect seedling success?

3.2 MATERIALS AND METHODS

3.2.1 Study species

Alliaria petiolata is an obligate biennial herb that experiences a single reproductive episode in its second year before dying (Cavers et al. 1979). Seeds germinate in early spring and grow into rosettes over the summer, fall and winter of the first year (Cavers et al. 1979). The rosette retains green leaves through out the winter months and is able to continue photosynthesis at a reduced rate (Dudley, personal communication). In the spring of the second year the rosette produces a single thin straight stem that bolts quickly into a tall slender stalk (raceme) with small white flowers. The lack of side branches and the straightness of the stalk suggest strong apical dominance. Flowers last between two and three days and will self-pollinate if not pollinated by visiting insects after the second day. The whole flowering period lasts between 4 to 6 weeks, by which time fertilized flowers produce slender fruit (siliques) containing up to 30 seeds each (Cavers et al. 1979). In southern Ontario, *A. petiolata* is found growing in large dense stands in disturbed sites and encroaching into deciduous forest understory.

3.2.2 Field observation of pollinator visitation

Two sites some 300 m apart were selected near the boathouse garden property of the Royal Botanical Gardens, in Hamilton, Ontario, Canada. At each site a plot of 2.25 m^2 within a natural stand of *A. petiolata* was marked off and insect pollinators were observed. For each site I recorded the height from the ground of visited flowers for as many as 4 stops on a pollinator's trajectory through the plot, after which pollinators were collected and identified to family and morphospecies. Pollinators were counted as having visited a floral display each time they landed on any of the open flowers on a flora cluster. Height measurements were taken using a series of vertical poles erected around the plot with centimetre markers such that one could accurately attain floral heights by following an imaginary horizontal line between any two poles and the floral display visited. Pollinator observations were carried out at both sites for 1 hour each time on 3 separate days during peak flowering in late May. After pollinator observations were concluded, all plants were systematically pulled out of the ground and a measurement from the base of each plant to each floral display was taken for every plant in each plot. From this I calculated the population mean floral display height, population plant density and the mean height of floral displays visited by pollinators. There were no significant differences between the two field sites in plant density, population floral display height, pollinator visited floral display height, or pollinator diversity, so data were pooled.

3.2.3 Height manipulation experiment

In early spring of 2002, second year rosette plants of *Alliaria petiolata* were collected from a natural population on the North shore properties of the RBG and transplanted into Styrofoam cups (10 cm diameter x14 cm tall). Transplants were taken to the boathouse garden at the RBG to first acclimate and then randomly placed into one of three artificial height treatments before bolting: tall, medium, and short. One hundred plants were placed into each of three treatments. Non-elevated treatment plants (short treatment) in cups were placed as is at 14 cm above the ground. Medium treatment plants were placed atop inverted pots such that the base of the plants were 21 cm from the ground and elevated treatment (tall treatment) plants were similarly placed such that the plants were 28 cm above the ground. Pots were placed in 10 rows of 30, each with a nearest neighbour distance of 10 cm. The density of plants, within the block, was 100 plants m^{-2}. This experimental plot was in direct sunlight from 9:30 a.m. to 6:30 p.m., and plants were watered every few days as needed to keep them moist.

True plant height (from base of stem to apex) was measured at the beginning of flowering and pollinators were allowed to forage undisturbed in the plot. Once flowering had ended, potted plants were removed from their treatments until fruit had matured. Plants were then collected and dried and measurements were taken for each plant for total plant mass, root mass, above ground stem mass, reproductive mass (total seed mass), total number of fruit, and total number of seeds. From this I calculated for each plant, average seed mass, average number of seeds per fruit, and vegetative mass (above ground mass minus reproductive mass).

3.2.4 Inbreeding depression

A. petiolata plants in their second year rosette stage were collected from the RBG property at Rock Chapel, Hamilton, Ontario, Canada in early spring of 2003 and transplanted into Styrofoam cups (10 cm diameter x14 cm tall) at the boathouse garden property of the RBG. Thirty plants were placed into each of three treatments in which plants were placed in individual cylindrical pollinator exclosures made from white mosquito netting and wire measuring 75 cm in height and 30 cm in diameter. Treatment 1 was a control group in which plants were placed in pollinator exclosures and left to self-pollinate. Treatment 2 plants were visited each day to be self hand-pollinated using fine forceps to place pollen from anthers onto stigmas of the same plant. Treatment 3 plants were visited each morning to be crossed-pollinated by hand using Q-tips to dust collected pollen onto individual flower stigmas. Self-pollination was assumed to be kept at a minimum as collected pollen was added each morning to newly opened flowers, which have yet to deploy their own mature stamens. Thus, the added pollen would pollinate all ovaries prior to any self-pollen being available. Fine forceps were used to collect stamens from flowers in a patch several kilometres away from the source of the experimental plants into a glass vial to be used on the same day. Thus, the vial used to pollinate treatment 3 plants contained a mixture of pollen from many different donors. Treatment plants were visited each morning weather permitting, between 9 am and 12:30 pm starting May 17/03 when flowers began to open. On June 16/03 all treatment plants no longer had any flowers and plants were removed from the exclosures and left in the field site for fruit to mature. On July 11/03 plants were harvested and air-dried several weeks. Once dried, data were recorded for above ground plant mass, total reproductive mass, vegetative mass, fruit mass, number of fruit, seed mass, and number of seeds. From this I calculated mean seed mass per plant, mean number of seeds per fruit, and mean fruit mass per plant.

From this seed set I selected 1600 seeds from each treatment. Outcrossed seeds came from 30 different families and ranged from 18 to 63 seeds per family selected randomly with a mean seed per family of 53.33 ± 2.17 (mean ± SE). Seeds of self-pollinated plants came from 30 different families with a range of 14 to 66 seeds per family selected randomly and a mean seed per family of 55.27 ±2.29 (mean ± SE). These seeds were randomly planted into 4 trays while maintaining a checkerboard pattern of alternating crossed and selfed seeds. Trays consisted of 20 X 40 1-cm^2 plugs filled with Promix BX soiless mixture. Seeded trays were placed into continuous darkness at 5 $^{\circ}$C on August 9, 2004, and were kept moist throughout their stratification period. Seeds began germinating November 18, 2004 at which point data were collected on date of germination. Trays were kept at 5 $^{\circ}$C until January 7, 2005 when they were placed under an 18:6 light/dark cycle at approximately 20 $^{\circ}$C. Seeds continued to germinate until January 20, 2005. All plants were collected from February 9 through February 11, 2005 and data were recorded for longest petiole length, largest leaf length, total leaf area, and total above ground dry weight. For each maternal plant, I calculated the fraction of seeds that did not germinate, the fraction that germinated, and those that germinated and survived to collection. These last two calculations I referred to as “germination” and “survival” throughout the thesis. For each seedling that survived I calculated neighbour density and excluded the perimeter rows. Neighbour density was calculated as the number of neighbours each plant had (from 1 to 8) and grouped into low medium and high neighbour densities.

3.2.5 Statistical Analysis

Statistical analyses were conducted using JMP version 4.0.4 (SAS Institute 1999) for Macintosh. For the natural pollinator observations, mean population floral display height and mean pollinator visited display height were normally distributed, so I used a one-way analysis of variance to determine if pollinators showed preference for taller floral displays.

Plant character measurements for the height manipulation experiment were normally distributed and so were not transformed. For the height manipulation experiment, effects of treatment, vegetative mass and their interaction on components of reproductive yield were determined by analysis of covariance (ANCOVA). I also examined the effects of elevation treatment and size on plant height with ANCOVA.

For the measurements of inbreeding depression on components of reproduction, I used ANCOVA with pollination treatment (selfed versus outcrossed) and maternal plant size as covariates.

In the seed germination and competition experiment, I analysed traits both using the maternal plant as the observation, and using the individual seedlings as the observation. I used seed mass as a covariate in both types of analysis, but because seed mass was measured as an average per family, all individuals within a family have the same average seed mass value in the seedling analysis. For individual seedlings, I analysed the effects of pollination treatment, neighbour density, average seed mass and the pollination treatment × average seed mass interaction on days to germination, hypocotyl length, petiole length, maximum leaf length, total leaf area, and above ground dry mass. For families, I determined the effects of pollination treatment, average seed mass and mother vegetative mass on days to germination, mean hypocotyl length, germination fraction, survival fraction, and total maternal fitness (calculated as survival fraction × number of seeds × mean seedling mass). To examine if there was a difference in germination between treatments, I used an ANCOVA with germination fraction as the independent variable and mean seed mass as covariate. Type III sums of squares were used for all analysis of variance (ANOVA) and covariance (ANCOVA).

3.3 RESULTS

3.3.1 Field study

The two field plots of *A. petiolata* had an average density of 142 ± 15 plant m^{-2}, with a mean floral display height of 65.95 ± 1.04 cm. The mean height of pollinator visited floral displays (89.8 ± 2.02 cm) was significantly taller than that of the mean height of floral display of the natural population census (Figure 3.1), indicating pollinators preferentially visited taller floral displays. The pollinator assemblage was dominated by small bees of which helictids were the most common (Table 3.1). One honey bee and one bumble bee (Family Apidae) were collected at one of the sites and included in the count but they were likely not pollinators as they only hovered over flowers and landed only briefly (Table 3.1). Unlike the Apidae, morphospecies of all other families were small, often less than 5 mm in length.

3.3.2 Height manipulation experiment

Pollinators were observed at the experimental plant array on several days. However, the number of pollinator visits observed was too low for a statistically significant analysis of pollinator preference to be carried out.

Analysis of covariance revealed that elevation treatment had a significant effect on final plant height ($n = 183, F = 7.48, P = 0.0007$). Non-elevated treatment plants elongated significantly more than elevated treatment plants ($F = 8.04, P = 0.005$), indicating a density effect on non-elevated treatment plants (Figure 3.2). Vegetative mass had a highly significant and positive effect on final plant height ($F = 259.11, P <$

0.0001). As plant size increased so did the difference in elongation between treatments (Figure 3.2).

There were no significant differences in reproductive output in *Alliaria petiolata* due to height manipulation treatment (Table 3.2). Treatment had no effect on flower, fruit or seed number (Table 3.2). However, elevated plants had significantly more seeds per fruit than non-elevated treatment plants (Table 3.2), suggesting that elevated treatment plants had higher pollination rates then non-elevated plants. Vegetative mass had a highly significant and positive affect on total seed mass, number of flowers, number of fruit, and number of seeds (Table 3.2). Neither elevation treatment ($F = 0.057$, $P = 0.811$, $df = 1$) nor plant vegetative mass ($F = 2.04$, $P = 0.156$, $df = 1$) had any significant effect on mean seed mass.

3.3.3 Hand pollination experiment

There were no significant differences in total seed number, seed mass or seed size between selfed and outcrossed hand pollinated plants (Table 3.3). Vegetative mass had a highly significant and positive effect on total seed mass, number of seeds, and number of fruit (Table 3.3). Mean seed mass and seed per fruit were not significantly affected by either pollination treatment or vegetative mass (Table 3.3).

3.3.4 Germination and seedling competition experiment

For the subset of seeds chosen for the germination experiment, selfed seeds were slightly larger than outcrossed seeds, but this was not a statistically significant difference

(Figure 3.3). A one-way analysis of variance revealed that seeds that germinated were significantly larger than those that did not germinate ($F = 299.41$, $P < 0.0001$), regardless of pollination treatment.

Differences between pollination treatments were more evident in early seedling traits than in later traits. Outcrossed treatment seeds germinated significantly sooner than selfed seeds (Table 3.4, Figure 3.4) and had significantly longer hypocotyl lengths than selfed seedlings (Table 3.4). Family level analysis revealed the germination fraction was higher for outcrossed families then for selfed families for any given seed mass, and as family average seed mass increased so did the germination fraction, and the difference in germination success between pollination treatments (Table 3.5, Figure 3.5). Outcrossed families also germinated sooner, on average, then selfed families (Table 3.5). Family average seed mass had a highly significant and negative effect on mean days to germination (Table 3.5). Germination fraction was negatively correlated with mean days to germination for both selfed and outcrossed plant families (Figure 3.6). Maternal vegetative mass had a significant and negative effect on mean days to germination, and a significant and positive effect on germination fraction, but no significant effect on hypocotyl length (Table 3.5). Neighbour density had a strong positive and significant effect on hypocotyl length (Table 3.4).

For later stage traits, effects of both average seed mass and pollination treatments were small or absent. Seedlings from selfed plants had weakly significantly longer maximum leaf lengths and total leaf area than outcrossed treatment seedling (Table 3.4). Surprisingly, there was no difference in above ground dry mass, or petiole length between selfed and outcrossed seedlings (Table 3.4). Cross-pollinated families had significantly higher seedling survival than selfed families, yet total maternal fitness (calculated as survival × number of seeds × mean seedling mass) did not differ significantly between pollination treatments (Table 3.5). Average family seed mass and maternal family vegetative mass both had highly significant and positive effects on survival fraction and total maternal fitness, but had no significant affects on mean petiole length, mean maximum leaf length, mean total leaf area, or mean seedling mass (Table 3.5).

Neighbour density had a strong significant effect on all later seedling traits. As neighbour density increased, maximum leaf length, total leaf area, and seedling dry mass decreased (Table 3.4). Petiole length was significantly affected by neighbour density and increased from low to medium and then decreased at high density (Table 3.4).

3.4 DISCUSSION

These studies provide support for the hypothesis that height effects plant fitness through increased pollinator attraction. However, maternal size and seed size appear to be the more important factors contributing to plant fitness in *A. petiolata*.

Field observations of pollinator behaviour showed that pollinator-visited floral displays were significantly taller than the average floral display height of the population, and in the experimental array, elevated plants produced more seeds per fruit then non-elevated plants, both suggesting that taller floral displays attract more pollinators. However, because of the low pollinator observations at the experimental array, I was not able to directly assess pollinator preference independent of size. However, in the closely related species *H. matronalis* pollinator visitation is highly correlated to plant height independent of plant size (Chapter 2).

Pollinator attraction will only be important if there is inbreeding depression. Because *A. petiolata* is a highly selfing species, it is likely that strongly deleterious recessive alleles would have been purged from the natural population and so effects of inbreeding depression may be due to mildly deleterious mutations which are more subtle and become evident only in early life stages (Husband and Schemske 1996). The greatest inbreeding depression was found among early seedling traits, where seeds of outcrossed families were more likely to emerge, emerged earlier, and had longer hypocotyl lengths then those of selfed families, given the same seed mass. Inbreeding depression was not evident in later seedling traits, with one exception; outcrossed families had significantly higher survival fractions then selfed families. All inbreeding depression had vanished by the time of harvest. However, this may be due to the low density in which plants were grown, and the lower sample size due to a high death rate. Had they been given more time to compete with one another in increasingly crowded conditions, seeds from outcrossed families may well have out competed those from selfed families through dominance and suppression. Potentially, inbreeding depression could be expressed in the field where population density is much higher than what was experienced in the germination experiment, a result that has been shown in *Impatiens capensis* (Schmitt and Ehrhardt 1990).

Early germination has been shown to correlate strongly with higher fitness in later life (Biere 1991). Early germination may be the principal mechanisms for long term success in *A. petiolata*, where seedling densities have been reported at 103 600 m^{-2} (Cavers et al. 1979), and where small differences in early germination and establishment may translate into significant fitness differences in later life stages as has been suggested for other monocarps (Sletvold 2002).

These results suggests that inbreeding depression is important in early seedling establishment but that the effects disappear at later stages, where seed size and maternal size appear to have much stronger effects. For example both maternal plant size and seed size had strongly significant and positive effects on predicted total maternal fitness, while pollination treatment showed no effect. This indicates that the role of floral display height in plant fitness is less important then that of plant size and seed size in the predominant self-pollinating species *A. petiolata*.

For individual seeds, size was of paramount importance, as seed size had a highly significant effect on days to germination, hypocotyl length, germination fraction, and survival fraction. Many other studies have shown similarly strong correlations between mean seed mass and germination, and speed of germination (Gross 1984, Stanton 1984, Sletvold 2002). Larger seeds have often been shown to have a competitive advantage over smaller ones (Gross 1984, Rees 1995, Weiner et al. 1997, Jakobsson and Eriksson 2000). In a study of wild radish, seedlings from larger seeds were found to have a reduced chance of dying and a higher reproductive output (Stanton 1985). However some researchers have shown no relationship between seed size and measures of fitness (Reader 1993), and others have shown the relationship to vary with the species studied (Basking and Baskin 1998).

In *A. petiolata*, seed size was the most important factor affecting all early seedling traits, seedling survival, and predicted maternal fitness; however, seed size did not correlate with maternal size. As plant size increased, seed number and total seed mass increased but mean seed mass did not change. This is consistent with findings in other *A. petiolata* studies (Susko and Lovett Doust 2000, Smith 2003), and some other monocarpic perennials (Gross 1984, Klinkhamer and de Jong 1987). As plant size increases, the gain in reproductive output is expressed as an increase in seed number rather than seed size, suggesting that seed number is more important then seed size to plant fitness (Schmid 1992). This result suggests a conflict between maternal fitness and individual seed fitness. Since individual seeds benefit from increased size but maternal fitness is determined by the sum fitness of all offspring produced. This may be a strategy to deal with ephemeral habitats where seed number is more important if seed mortality is high.

Maternal vegetative mass was found to decrease days to germination, and to increase germination fraction, survival fraction, and predicted total maternal fitness. Yet maternal vegetative mass had no effect on seed size. This suggests that larger maternal size provides some component of seed quality independent of seed size (Stearns 1992, Husband and Gurney 1998). In a study of the facultative biennial *Digitalis purpurea*, germination fraction, days to germination and seed mass were found to be positively correlated with maternal plant size, and the study concluded that increased plant size provided for increased offspring quality (Sletvold 2002). However, in the monocarpic perennial *Cynoglossum officinale*, no relation was found between maternal plant size and seed size or percent germination (Klinkhamer and de Jong 1987). In *A. petiolata*, maternal size had a significant but weak effect on days to germination and germination fraction, but a strong significant effect on survival fraction and total maternal fitness (calculated as survival × number of seeds × mean seedling size). This suggests that plant size in *A. petiolata* benefits plant fitness by providing some non-nuclear genetic advantage and by increasing seed numbers. Thus, effects of maternal size are more important then the effects of flora display height on overall fitness.

The effects of competition on seed germination and early seedling development may be a key factor promoting outcrossing in this highly self-pollinating species. Because *A. petiolata* often germinates in very high densities, competition at the earliest stages of life should lead to marked differences in development, where small differences in seed quality should be magnified by intense competition for nutrients, space and light (a mechanism know as dominance and suppression). In this study, density strongly affects all traits of early growth. High-density areas produced seedlings with lower mean leaf length, leaf area and seedling mass, suggesting there was competition for resources. In response to increased density, seedlings produced longer hypocotyls, which can be interpreted as a competition response to density, since a longer hypocotyl allows a seedling to develop above its neighbours, thus, out competing them for light and space (Sanchez 2002). In the germination experiment there was no difference in response to density between treatments. However because density was low due to low germination and high mortality, these results should be viewed with caution. Furthermore, this result is inconsistent with findings by other researchers that found fitness difference between outcrossed and selfed plants were intensified by dominance and suppression under high density (Schmitt and Ehrhardt 1990).

Self-pollination in species with low inbreeding depression may be adaptive in low-density populations and in early colonization, promoting high seed output and fast colonization in the absence of pollinators. But as density increases, outcrossing becomes more important because it provides the genetic diversity advantageous in a highly competitive environment. Heterozygosity in self-pollinating species has often been observed to be higher than expected on the basis of mating system, suggesting a persistent fitness advantage to outcrossing (Sanders and Hamrick 1980, Schemske and Lande 1985). To this end, density-dependent stem elongation may provide a mechanism by which a plant can maximize its chances of outcrossing.

In the height manipulation experiment, elevation treatment affected stem elongation. On average non-elevated treatment plants grew significantly taller than elevated treatment plants. This is consistent with other studies in which *A. petiolata* was found to elongate in response to growth in high density stands (Meekins and McCarthy 2000, Sanchez 2002). Density-dependent stem elongation was also found in other herbaceous species and was shown to provide fitness advantages in high-density stands (Dudley and Schmitt 1996). Density dependent stem elongation has often been explained in terms of competition for carbon acquisition (Dudley and Schmitt 1996, Sanchez 2002); however elongation could potentially play an important role in pollinator attraction.

Observation of the height manipulation experiment confirmed a relatively low frequency of pollinator visits overall to the experimental array as compared to observations of natural *A. petiolata* stands. Thus, elevated plants in the height manipulation experiment may have had much higher rates of self-pollination than expected had they been frequently visited by pollinators, reducing the contrast in pollination regimes that I had assumed would come about due to pollinator behaviour. The large distance between the experimental arrays and any source of potential pollinators such as forest edge or old-field, where pollinators were seen in abundance, may have caused the low pollinator abundance at the experimental array.

This study contradicts the findings of Susko and Lovett-Doust (2000) who found that smaller seeds germinated significantly earlier among *A. petiolata* from Ohio. In this study larger seeds germinated earlier and more frequently then smaller seeds. In their experiment, seeds were kept in petri dishes at 1 °C in darkness for stratification and then planted, prior to breaking dormancy, into soil mixture pots kept at 20 °C and a 14/10 light to dark cycle. In this experiment, seeds were placed in soil from the very beginning and kept in a dark, cold (5 °C) chamber well after first germination with only occasional exposure to light while germination was being assessed. This experiment more closely approximated natural conditions for *A. petiolata* in S. Ontario, in that seeds begin germinating very early in the spring under cold temperatures and often in semi darkness under snow cover (S. Irazuzta, personal observation). In favourable years when seeds begin germinating in early spring without snow cover, early germination could produce large fitness differences, since early geminated seeds would out compete later germinated seeds for light and space. It may be that the colder conditions during early germination and growth in this experimental design may account for the lack of size difference between pollination treatments, and that cool temperatures and lack of light dampened the benefits of early germination on seedling size. Differences in germination techniques, and regional variation in seed stock may account for the difference in these results from those of Susko and Lovett-Doust (2000).

Table 3.1. A summary of insect pollinator diversity for two natural stands of *Alliaria petiolata*.

	Site 1		Site 2	
Families	Number of morphospecies	Number of individuals	Number of morpho-species	Number of individuals
Bees – total	12	34	10	36
Andrenidae	3	7	3	9
Anthophoridae	2	6	2	6
Helictidae	5	17	5	21
Apidae	2	2	0	0
Flies				
Syrphidae	3	4	3	6
Total	15	38	13	42

Table 3.2: An analysis of covariance for fitness traits in elevated and non-elevated treatment plants of *Alliaria petiolata* visited by a natural pollinator assemblage. The model was not significant for seed size. No interaction effects were significant and so are not reported. *P* values are given in parenthesis.

Source	*df*	*F*-ratios				
		Total seed mass $n = 129$	Number of seeds $n = 129$	Number of fruits $n = 182$	Number of flowers $n = 182$	Seeds per fruit $n = 129$
Elevation treatment	2	0.0002 (0.99)	0.60 (0.44)	0.0074 (0.93)	1.42 (0.24)	5.66 (0.019)
Vegetative mass	1	817.64 (<0.0001)	708.67 (<0.0001)	1103.19 (<0.0001)	134.56 (<0.0001)	6.61 (0.011)

Table 3.3. Analysis of covariance for reproductive traits in artificially self-pollinated and cross-pollinated treatment plants of *Alliaria petiolata*. No interaction effects were significant and so were omitted. The overall model was not significant for mean seed mass or seed per fruit. $N = 50$.

Source	*df*	*F*-ratios				
		Total seed mass	Number of seeds	Mean seed mass	Number of fruits	Seed per fruit
Pollination treatment	2	0.7251 (0.3988)	0.0202 (0.8875)	2.0281 (0.1610)	0.4571 (0.5023)	0.7833 (0.3806)
Vegetative mass	1	33.7937 (0.0001)	51.1418 (0.0001)	0.1018 (0.7511)	45.3015 (0.0001)	5.4322 (0.2410)

Table 3.4. Analysis of covariance for fitness traits in competing seedlings from outcrossed and self-pollinated plants of *Alliaria petiolata*. All traits were measured on each seedling, except average seed mass, which was the family mean. $N = 520$.

Source	*df*	*F*-ratios					
		Days to germination	Hypocotyl length	Petiole length	Max. leaf length	Total leaf area	Above ground dry mass
Pollination treatment	1	15.7443 (0.0001)	4.4671 (0.0350)	1.0810 (0.2990)	4.2372 (0.0401)	4.0407 (0.0449)	2.9898 (0.0844)
Neighbour density	2	8.5439 (0.0002)	24.2487 (0.0001)	7.1043 (0.0009)	4.8414 (0.0083)	9.5731 (0.0001)	7.1669 (0.0009)
Average seed mass	1	11.0664 (0.0009)	24.0670 (0.0001)	3.4285 (0.0647)	0.0001 (0.9925)	0.0179 (0.8935)	1.2983 (0.2551)
Treatment × Average seed mass	1	5.3265 (0.0214)	0.5307 (0.4667)	4.6849 (0.0309)	6.0779 (0.0140)	4.6427 (0.0317)	4.8829 (0.0276)

Table 3.5. ANOVA of the effects of treatment, average seed size, and plant vegetative mass on family mean traits of artificially outcrossed and self-pollinated plants of *Alliaria petiolata*. *P* values are given in parenthesis. The overall model was not significant for petiole length, maximum leaf length, total leaf area, or seedling mass, so results are not shown. No interaction effects were significant and so omitted. Total maternal fitness was an estimate calculated as survival fraction × number of seeds × mean seedling mass. *N* = 55.

Source	*df*	*F*-ratios				
		Germination fraction	Mean Days to germination	Mean hypocotyl length	Survival fraction	Total maternal fitness
Pollination treatment	1	8.3697 (0.0056)	4.8111 (0.0329)	4.1446 (0.0472)	5.6873 (0.0208)	2.0020 (0.1634)
Average seed mass	1	140.9948 (0.0001)	15.9992 (0.0002)	11.9478 (0.0011)	65.4446 (0.0001)	23.6947 (0.0001)
Maternal vegetative mass	1	4.7239 (0.0344)	5.6285 (0.0215)	0.6847 (0.4120)	9.6092 (0.0031)	61.7893 (0.0001)

Figure 3.1. Mean floral display height of a natural population of *A. petiolata* plants (*n* = 641, mean ± SE= 65.95 ± 1.04 cm) and the mean height of insect pollinator visited floral displays (*n* = 80, mean ± SE = 89.8 ± 2.02) within that population. Heights were statistically different from each other (one-way ANOVA $F = 40.70$, $P < 0.0001$, df=1). Bars represent 1 SE.

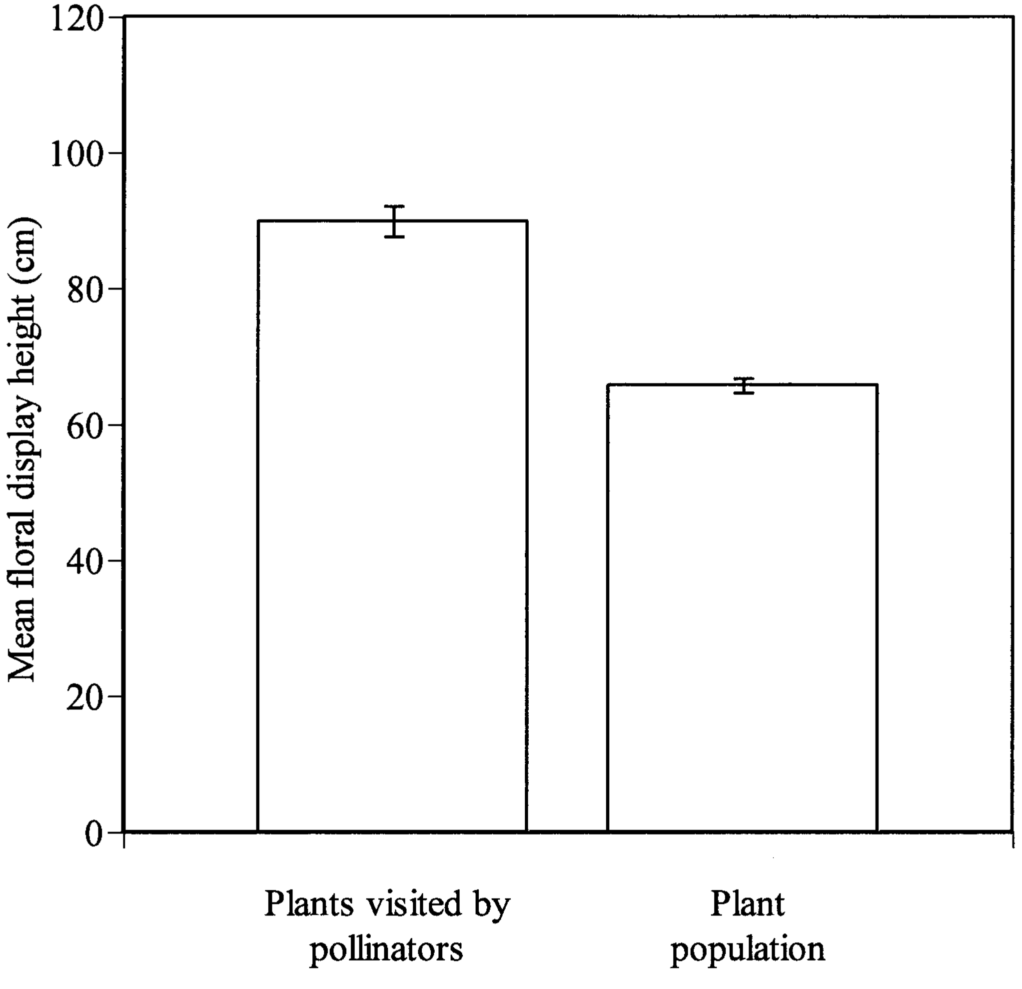

Mean floral display height (cm)
120
100
80
60
40
20
0
Plants visited by pollinators
Plant population

Figure 3.2. Final plant height as a function of plant vegetative mass in experimental plants of *Alliaria petiolata*. Plant height increases with mass at a higher rate for non-elevated treatment plants (solid line, linear regression is: $y = 29.28x + 32.47$, $r^2 = 0.68$, $n = 87$) than for elevated treatment plants (dotted line, linear regression is: $y = 20.51x + 35.75$, $r^2 = 0.52$, $n = 96$). Analysis of covariance revealed the interaction to be significantly different indicating elongation in non-elevated plants ($F = 8.04$, $P = 0.005$).

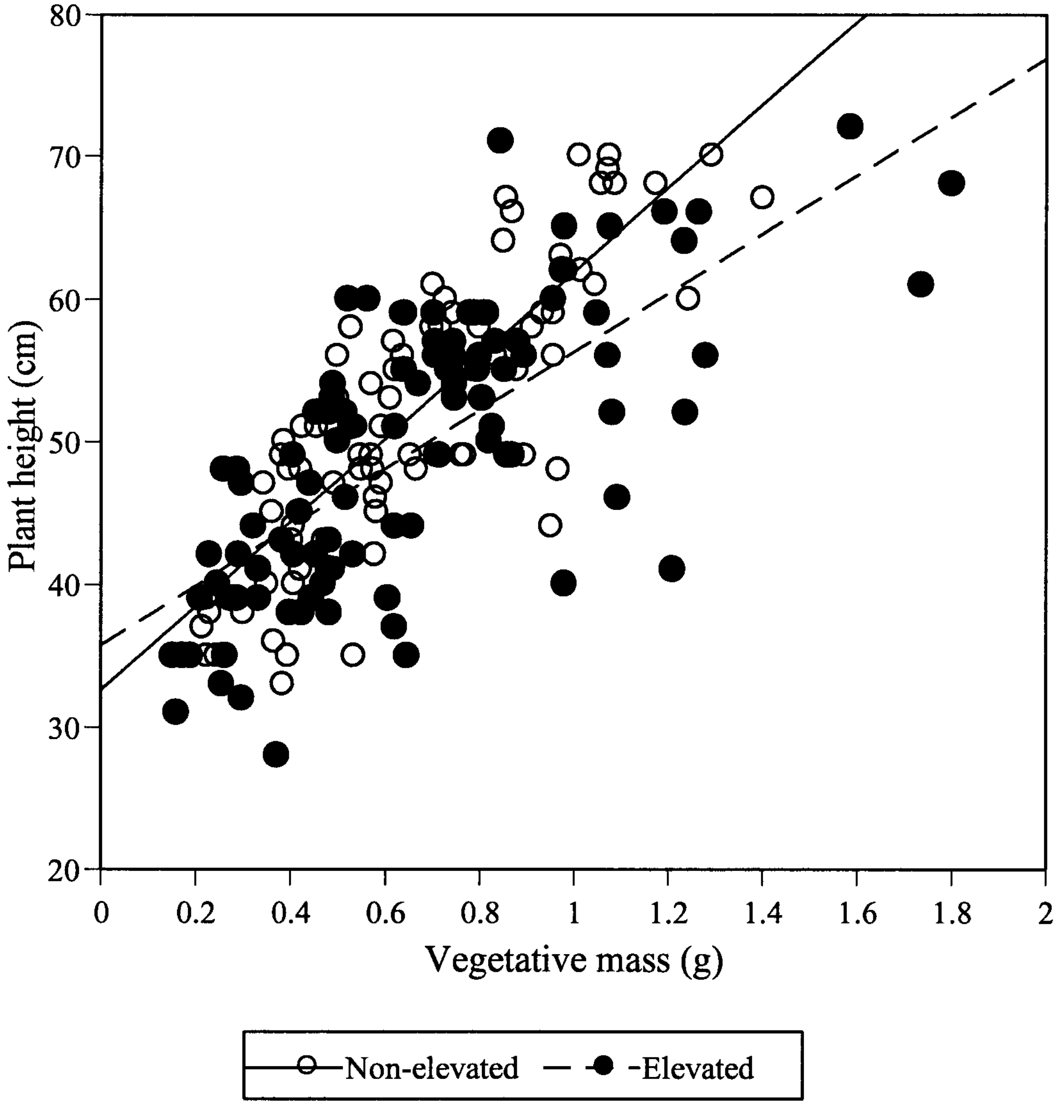
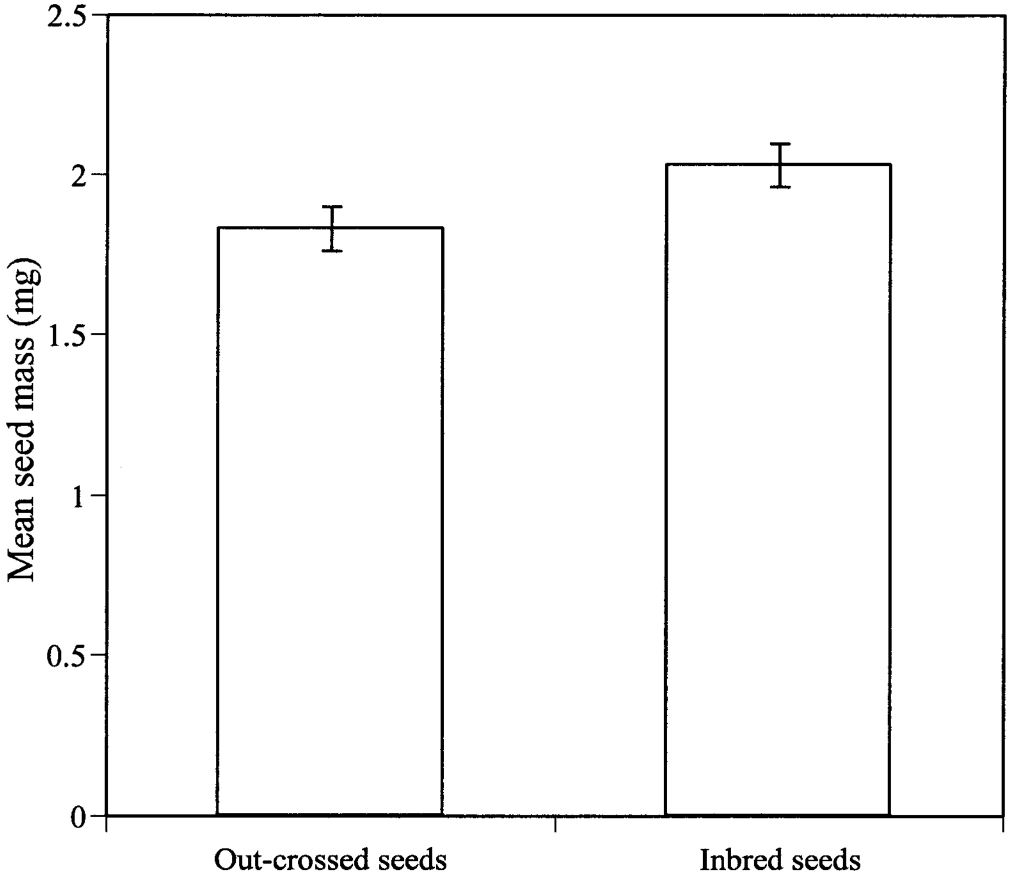

80
70
60
50
40
30
20
Plant height (cm)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
Vegetative mass (g)
Non-elevated
Elevated

Figure 3.3. Family mean seed mass of seeds produced by artificially outcrossed and inbred treatment plants of *Alliaria petiolata*. There was a nearly significant difference between treatments (One-way ANOVA, $F = 3.68$, $P = 0.06$, $df = 1$). $N = 55$. Bars indicate 1 SE.



Mean seed mass (mg)
2.5
2
1.5
1
0.5
0
Out-crossed seeds
Inbred seeds

Figure 3.4. Days to germination for *Alliaria petiolata* seeds resulting from cross-pollination ($n = 718$) and self-pollination ($n = 727$). Seeds resulting from outcrossing germinated significantly earlier than seeds from self-pollination. See Table 3.5.

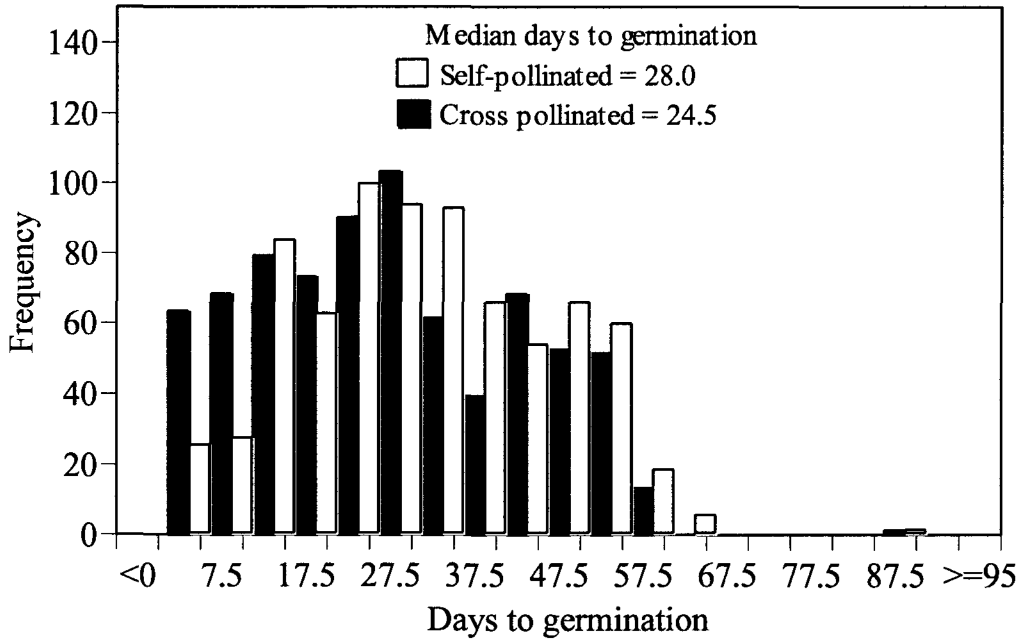

Median days to germination
☐ Self-pollinated = 28.0
■ Cross pollinated = 24.5
Frequency
0
20
40
60
80
100
120
140
<0
7.5
17.5
27.5
37.5
47.5
57.5
67.5
77.5
87.5
>=95
Days to germination

Figure 3.5. Fraction of seeds that germinated versus mean seed size for cross-pollinated (dotted line) and self-pollinated (solid line) families of *Alliaria petiolata*. Outcrossed plants had significantly higher seed germination than self-pollinated plants ($n = 54$, $df = 1$, $F = 7.29$, $P = 0.0094$). Cross-pollinated least squares means (LSM) = 0.537, mean ± SE = 0.476 ±0.025, Inbred LSM = 0.441, mean ± SE =0.488 ± 0.026.

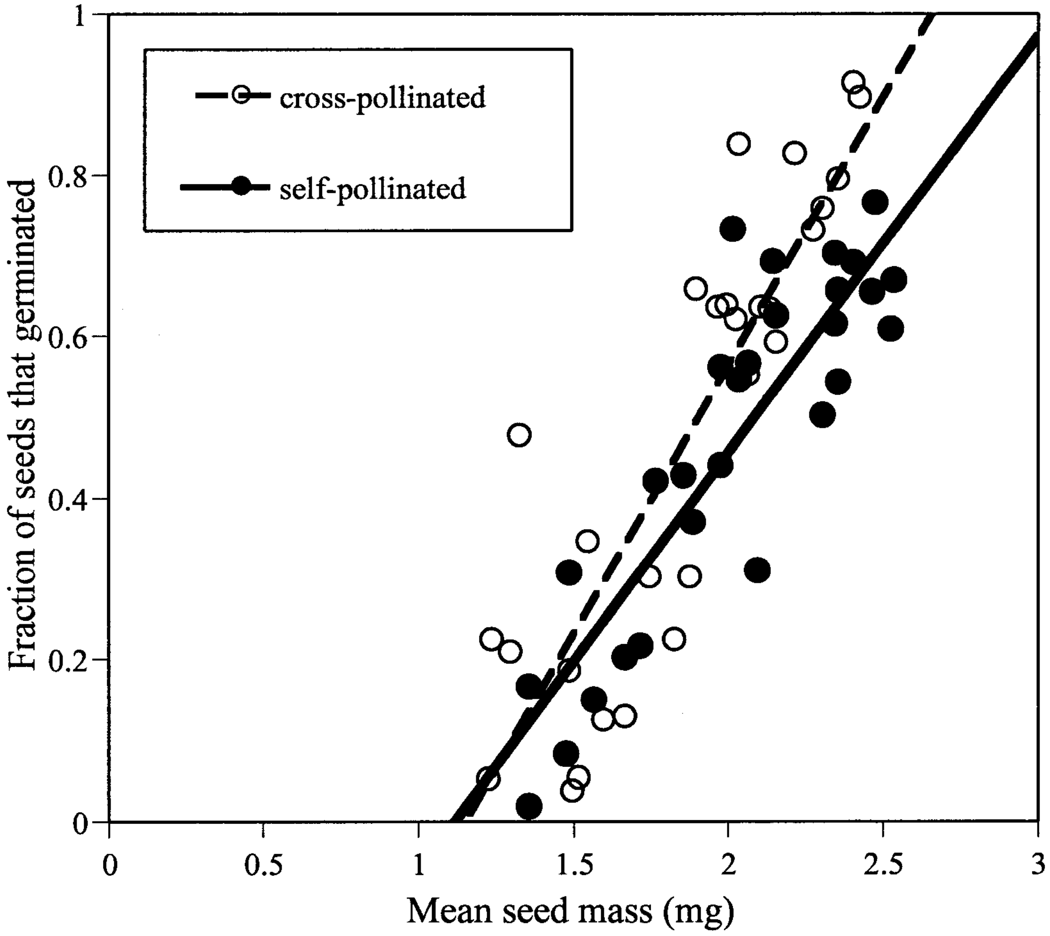
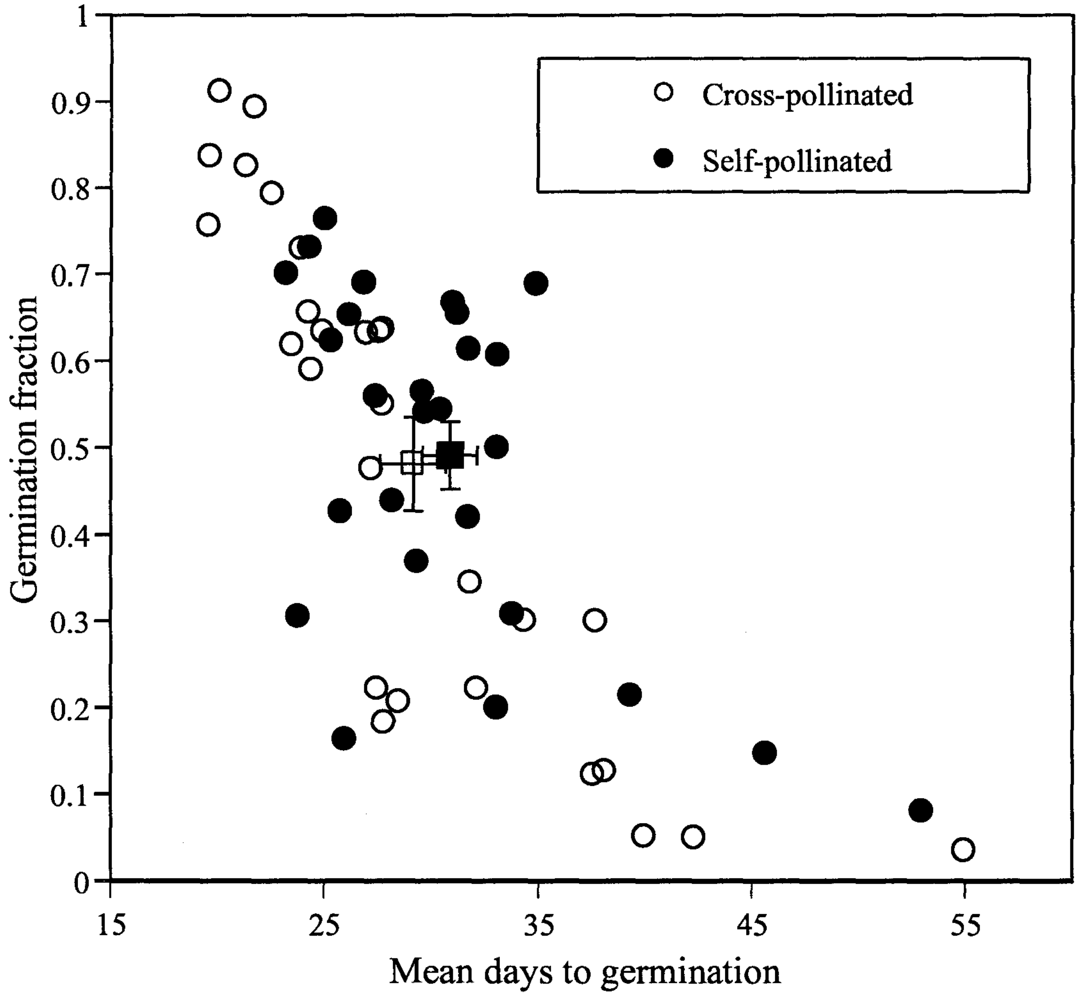

cross-pollinated
self-pollinated
Fraction of seeds that germinated
0
0.2
0.4
0.6
0.8
1
Mean seed mass (mg)
0
0.5
1
1.5
2
2.5
3

Figure 3.6. A scatter plot of the fraction of seeds that germinated as a function of mean days to germination for artificially cross-pollinated (open circles) and selfed (closed circles) families of *Alliaria petiolata.* A significant negative correlation exists for both treatments. Cross-pollinated plants $n = 28$, $r^2 = -0.84$, $P < 0.0001$; Self-pollinated plants, $n = 27$, $r^2 = -0.59$, $P = 0.0013$. The open box and the closed box with horizontal and vertical bars indicate mean ± SE for days to germination and germination fraction for cross-pollinated and selfed plants respectively.


Germination fraction
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
15
25
35
45
55
Mean days to germination
○ Cross-pollinated
● Self-pollinated

CHAPTER 4

SUMMARY

In the previous chapters I investigated the role of floral display height as pollinator attractant and the consequences of inbreeding in two quite different mating systems within two closely related monocarpic species. The importance of floral display height in attracting pollinators independent of plant size was clearly demonstrated experimentally for *H. matronalis* and inferred for *A. petiolata* through observation of pollinator behaviour in natural populations.

The consequence to maternal fitness of increased pollinator visitation was shown to differ between mating systems. In chapter 2, I demonstrated that differences in pollinator visitation bring about significant differences in allocation to reproductive output in a predominant outcrosser. *H. matronalis* was shown to exhibit a clear trade off in reproductive allocation between producing many small seeds in elevated treatment plants and few large seed in non-elevated plants. Since increased seed size is highly correlated to higher fitness traits (Gross 1984, Weiner et al. 1997) (i.e. faster germination, higher survival) it would stand to reason that maximum fitness would be achieved by producing many large seeds. However, total parental fitness is ultimately dependent on how well all offspring do, as measured by their total combined growth and seed production. Thus, it may be an evolutionarily sound strategy to produce many more small seeds that have lower germination and survival fractions but that, overall, produce more progeny and overall more seeds than a few successful large offspring (Sakai and Sakai 2005). By producing more, smaller seeds the parent is also increasing the chances of survivorship from random destructive events that may occur. Producing many small seeds also reduces the over all effect of seed predation. For example, seed predation may be more intense among large seeds since they provide more food per unit than smaller seeds (Janzen 1970, Gomez 2004). Also, many small seeds are more likely to fall further from the mother plant than are few large seeds, increasing the opportunity to colonize new habitat where intraspecific competition may be lower (Ezoe 1998).

In contrast, *A. petiolata* showed no difference in reproductive allocation (as measured by total seed mass, seed number and mean seed mass) in response to floral display height. Even when pollinator abundance was simulated by artificially outcrossed and self-pollinated treatments, only trends in differing maternal allocation were noticed. Nevertheless, there were measurable differences in seed quality as measured by percent germination, time to germination and survival, and between seeds of outcrossed and inbred plants of *A. petiolata,* indicating that inbreeding depression persists even in this highly inbreeding species. In this case, even though pollinators preferred taller floral displays, pollinator-mediated selection is diminished first by the tendency to self-pollinate and second by the lower magnitude of inbreeding depression.

Maternal plant size had no effect on mean seed size in *A. petiolata,* yet there were significant differences in germination, time to germination, and survival in comparison to maternal size, suggesting that maternal investment in seed cannot be measured by mean seed mass alone. For *A. petiolata* larger individuals appear to produce seeds of higher fitness independent of the degree of outcrossing, or seed size.

Unlike other monocarpic perennials, neither *H. matronalis* nor *A. petiolata* showed an effect of maternal size on seed size, suggesting seed size is a conservative trait in these two species. Thus, the increased resources available to larger individuals were allocated to producing more seeds and not larger seeds. However, at the level of the individual seed, size was the most important factor affecting fitness in *A. petiolata* indicating a possible conflict between maternal fitness and individual seed fitness.

My results support the idea that floral display height as a component of plant size can increase plant fitness not only by directly increasing seed numbers by attracting more pollinators, as seen in *H. matronalis*, but also by increasing seed fitness through increased outcrossing as seen in *A. petiolata*. For *A. petiolata*, differences in seed quality can be due to genetic differences brought about by pollinator-mediated selection or though maternal size. Surprisingly, despite the fact that outcrossed seedlings germinated sooner and in higher numbers, total maternal fitness did not vary between pollination regiments. This may have been caused by a lack of strong competition pressure, which had been planned to occur in the high-density germination experiment. However, this was diminished by the overall low percent germination and the patchy nature of seed germination in this study. Such limitations could be corrected in future studies with more careful selection of the germination medium and humidity controls.

In both species, larger plants tended to emphasize the female function, since larger plants produced more seeds per fruit, presumably from higher pollination frequency, yet they produced proportionally fewer fruit. This suggests that smaller plants promote pollen export while larger plans emphasize seed production.

In these experiments, it was found that *A. petiolata* elongated in response to density, while *H. matronalis* did not, despite the fact that both were grown at equal densities of 100 plants per m^2. This phenotypic plasticity in *A. petiolata* may have evolved in response to strong selection for carbon acquisition in a species that is often found in very high densities relative to *H. matronalis*. *A. petiolata* field sites had a density of 127 plants per m^2 while *H. matronalis* field sites had a density of 35 plants per m^2. Presumably germination densities are much higher than this.

To conclude, these studies have revealed the mechanisms through which plant size can regulate seed quality. Floral display height as a component of plant size promotes an increase in plant fitness by increasing seed quality via increased pollinator attraction and thus greater outcrossing and reduced pollen limitation. While increased maternal size, promotes higher fitness by increasing maternal investment in both seed quantity and non-nuclear genetic quality. Finally, comparison of the two species indicates that mating system clearly affects the importance of floral display height in plant fitness, where lower inbreeding depression reduces the selection pressure for outcrossing in *A. petiolata*.

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