

THE UNIVERSITY OF CALGARY

Limitations to Reproduction in Monkshood, *Aconitum*
delphinifolium, and Tall Larkspur, *Delphinium glaucum*

by

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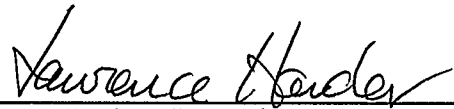
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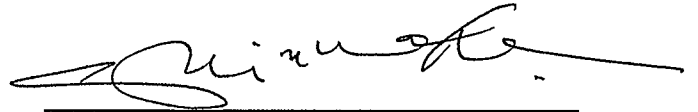
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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Limitations to Reproduction in Monkshood, *Aconitum delphinifolium*, and Tall Larkspur, *Delphinium glaucum*" submitted by Michael B. Vander Meulen in partial fulfillment of the requirements for the degree of Master of Science.



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ABSTRACT

Monkshood, *Aconitum delphinifolium* DC (Ranunculaceae), and tall larkspur, *Delphinium glaucum* S. Wats. (Ranunculaceae) flower sympatrically with considerable phenological overlap. Consequently, their seed production could be limited intraspecifically, because of inadequate pollination or insufficient resources, or interspecifically, because of competition for pollinators or interspecific pollen transfer. Both species are self-compatible but pronounced protandry ensures predominant outcrossing. Larkspur experienced inbreeding depression. Multiple pollen donors did not affect reproductive success. Reproductive interference between larkspur and monkshood through interspecific pollen transfer was not detected. Natural levels of interspecific pollination are insignificant. Heterospecific pollination reduced seed set, but simultaneous conspecific and heterospecific pollination had no effect. Simultaneous resource enhancement and supplemental pollination determined that maternal success in larkspur was limited by soil phosphorus levels. Fruit and seed production by monkshood are equally limited by resources and pollen receipt. Seed abortion and seed size and number trade-offs are discussed in terms of resource and pollen limitation.

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1.1 Factors limiting plant reproduction

Limitations to fruit and seed set are readily apparent; not all flowers develop into fruits and not all ovules set seed (Lloyd *et al.* 1980, Sutherland 1986, Wiens *et al.* 1989). Limited fecundity has short- and long-term implications for plants. Lowered fecundity will result in reduced representation in subsequent generations and possible elimination of populations (Levin and Anderson 1970). Reproductive limitations may also have evolutionary consequences for floral biology. If limited fecundity is caused by inadequate nutritional resources then the maternal sporophyte will be selected to mature zygotes representing the best fitness prospects (Bertin 1982a, Stephenson and Bertin 1983) and conversely, male function will be selected for more efficient pollen dissemination (Johnston 1991a). Conversely, insufficient pollen receipt may result in selection for flowers that are receptive longer and promote pollen receipt (Stephenson and Bertin 1983, Johnston 1991a).

Sexual reproduction in plants is a complex process that depends on a variety of component processes, so that reproduction could be limited at any of several stages. In general, sexual reproduction may be limited by pollen quantity, pollen quality or other resources (Zimmerman and Pyke 1988). After production of gametes, the first step in sexual reproduction is pollen transfer from male to female structures. However, insufficient pollination means that not enough pollen grains are available to fertilize all ovules. In addition to insufficient pollination, reproductive success may be affected by the quality of pollen received; pollination must be by conspecific pollen of suitable genetic quality. Following successful fertilization of ovules, maternal success depends on the ability of plants to adequately nourish developing fruits and the seeds they contain. Shortages of nutritional resources at this stage may result in abortion of developing seeds or of whole fruits.

1.1.1 Limitations on pollen receipt

Insufficient pollen receipt results in fewer ovules being fertilized than the flower is capable of developing into seeds, and has been observed for many plant species (Bierzychudek 1981, Campbell 1987, Johnston 1991b, Karoly 1992).

Limiting levels of pollination may result from infrequent pollinator visitation.

Inadequate pollinator visitation may be caused by environmental factors that interfere with pollinator movements and behavior (Campbell 1987). Pollen limitation may also be caused by the usurpation of pollinators from one species by another. Sympatric plant species may compete for the attention of animal pollinators (Levin and Anderson 1970) and this competition may affect reproductive success (Campbell 1985, Campbell and Motten 1985, Waser 1978a, 1978b).

1.1.2 Pollination and successful fertilization

The genetic identity of pollen may affect reproductive success. Pollen transfer between different genets (xenogamy) is often selected for the avoidance of the ill effects of inbreeding. Inbreeding depression is the loss of plant vigor in homozygotes relative to heterozygotes (Charlesworth and Charlesworth 1987). Homozygosity facilitates the expression of recessive alleles that may be lethal or reduce plant vigor. Inbreeding depression may be expressed in reduced germination rates for seeds, lower survival probabilities for seedlings and lower competitive abilities of these seedlings (Richards 1986, Levin 1989).

Self-fertilization (autogamy if pollen donation is within a single flower and geitonogamy if pollen donation is between different flowers of the same genet) does not always have adverse effects and many species self-fertilize regularly. Self-fertilization may be advantageous because of the reproductive efficiency it confers (Richards 1986). Plants that self-fertilize are assured of reproductive success even if cross-pollination mechanisms fail (Jain 1976). This reproductive assurance may be

critical to the perpetuation of colonizing and widely-dispersed species that experience a shortage of potential mates (Jain 1976).

The genetic consequences of inbreeding may also be beneficial. Inbreeding will promote the fixation of adaptive genotypes (Richards 1986) and conversely, may purge recessive lethals as the resulting homozygosity exposes them to selection (Barrett and Charlesworth 1991). Thus inbreeding populations may be adapted to local conditions and experience high reproductive success at the cost of being less capable of adapting to changing environments (Jain 1976). In a population with both self-fertilizing and outcrossing individuals, the former has the advantage of being able to pollinate both itself and the xenogamous individuals.

Fertilization is preceded by pollination and plants exhibit an array of mechanisms by which the identity of the fertilizing pollen they receive may be controlled. In hermaphroditic flowers self-pollination may be prevented by dichogamy which is the temporal separation of male and female functions (Lloyd and Webb 1986); protandrous individuals function first as males and then as females, whereas in protogynous individuals stigma receptivity precedes pollen presentation (Bawa and Beach 1981, Lloyd and Webb 1986). Plants may also have physiological means of controlling fertilization. Self-incompatibility is a mechanism that permits fertilization of ovules by genetically compatible pollen only (de Nettancourt 1977).

Pollen reaching a stigma must meet the requirements of any incompatibility mechanisms. Compatible pollen grains must germinate and grow pollen tubes faster than any other grains if they are to fertilize the ovules. Some pollen tubes will grow faster than others through the stylar tissue and may be more successful in fertilization (Mulcahy 1974, Lee 1984, Marshall and Ellstrand 1986, Snow 1986, Winsor *et al.* 1987). Gametophytic selection may occur as compatible pollen grains from different

sources compete with each other (Stephenson and Bertin 1983) resulting in non-random mating (Marshall 1991).

Pollen that is effective in fertilizing ovules will be of a particular genetic quality. However, the effect of pollen genetic identity is not entirely separable from the receipt of sufficient quantities of pollen on stigmas. More pollen than is necessary for full fertilization may be placed on a stigma allowing the maternal plant to select among these potential sires via differential pollen tube growth or incompatibility mechanisms. Maternal fitness may be maximized if an optimal number of pollen donors are sampled (Janzen 1977).

1.1.3 Limitations to fruit set and seed set

Following successful fertilization of ovules the plant must allocate resources to the developing seeds and fruits. The total seed and fruit set may be limited by the quantity of nutrients that are available for seed and fruit maturation (Stephenson 1980, McCall and Primack 1985, LaLonde and Roitberg 1989).

Although resource limitation may be proximately responsible for failed fruit and seed set, both pollen and resource limitation may be responsible for overall reproductive success. If pollination is sufficient but resources are limiting, fertilized ovules will not necessarily mature because the maternal plant may cull offspring via seed or fruit abortion (Stephenson and Bertin 1983, Lee 1988). Abortion need not be random and fruit maturation may depend on the number and genetic quality of developing seeds (McDade and Davidar 1984, Becerra and Lloyd 1992), and seeds within fruits may be selectively matured on the basis of their relative genetic quality (Temme 1986, Haig 1990).

Theoretical considerations suggest that plants will be selected to be equally limited by pollen receipt and resources (Haig and Westoby 1988a). The total reproductive effort by plants includes production of floral structures that promote

pollination (i.e. bright attractive flowers that offer rewards to pollinators) and the provisioning of developing seeds and fruits (Haig and Westoby 1988a). When pollen receipt and nutritional resources equally limit reproductive success, plants achieve the maximum fitness possible, given available reproductive resources. An allocation that promotes pollinator visitation to such an extent that the numerous fertilized ovules cannot be nourished would result in resource limitation; whereas the converse, insufficient pollen transfer, results in some resources spent on producing female structures being wasted.

This simple model proposes that plants experience selective pressure to be equally limited by pollen receipt and resources. However, this assumes that nutritional resources and pollen receipt are equally variable in consecutive reproductive seasons. It is unrealistic to assume that one factor will be consistently limiting between years and that a constant selective pressure will impinge on the plants, because pollen and resource availabilities are determined by many environmental influences. However, the model is certainly useful for examining reproductive limitations within a single season and determining the relative limitations imposed by nutritional resources and pollen receipt.

1.2 Objectives

In this thesis I examine the limits to seed production in monkshood, *Aconitum delphinifolium* DC (Ranunculaceae), and tall larkspur, *Delphinium glaucum* S. Wats. (Ranunculaceae). I characterize the protandry in each species because it is a feature that promotes outcrossing. I examine the possibility of interspecific interference for pollination during the period of phenological overlap because this may limit reproduction. I determine whether reproductive success is limited by pollen receipt or resource levels and discuss the implications of these results.

Specifically, in Chapter 2 I establish the self-compatibility of both species and determine the extent of any inbreeding depression using manual pollinations of flowers. Further manual pollinations will examine the effects of outcrossing and interspecific pollen transfer. Natural levels of outcrossing will be determined. Manual pollination with multiple donors will be performed and any effects determined. In Chapter 3 I determine whether reproductive success for each species within a single season is limited by pollen receipt or levels of available nutritional resources. This investigation involves supplemental pollination and resource enhancement treatments administered simultaneously. Additionally I determine whether there is any resource limitation expressed in seeds as a size-number trade-off.

2.1 Interspecific and intraspecific influences on the quality and quantity of pollen receipt and subsequent success of female function

Sexual reproduction in angiosperms is a complicated process which requires the union of gametes via pollination, pollen tube growth and fertilization. Obstacles to successful reproduction include insufficient pollination due to competitive interactions with other plants for pollination vectors and improper pollen transfer between species. Intraspecific pollination may be complicated by incompatibility mechanisms that limit fertilization on the basis of genetic quality.

This chapter will examine various aspects of the respective breeding systems of larkspur and monkshood. The results of pollination will be examined from the perspective of female reproductive success.

2.1.1 Breeding systems

The breeding systems of sexually reproducing plants promote receipt of sufficient quantities of appropriate pollen so that as many ovules as possible in a flower can be fertilized. This pollen must be from an appropriate source; whether pollen has the same genotype as the ovules it is attempting to fertilize may or may not matter. Many plants can reproduce successfully regardless of the origin of the conspecific pollen they receive and they self-fertilize regularly (Richards 1986); however, many other species experience inbreeding depression upon self-pollination (Charlesworth and Charlesworth 1987). Inbreeding depression is a loss of plant vigor in homozygotes relative to heterozygotes, whether that be due to the relative superiority of heterozygotes (overdominance) or the fixation of recessive alleles in homozygotes (partial dominance: Charlesworth and Charlesworth 1987). Inbreeding depression may be expressed as reduced seed set, fruit set, seed germination, survivorship, vigor or increased seed abortion during development and reduced

competitive ability of seedlings (Richards 1986, Levin 1989, Manasse and Pinney 1991).

Self-fertilization can be prevented through numerous means. To counter the negative effects of inbreeding depression, plants may have self-incompatibility mechanisms that prevent self-fertilization (de Nettancourt 1977). Self-incompatibility is a physiological mechanism that prevents the production of zygotes following self-pollination (de Nettancourt 1977). Depending on the species, self-incompatibility may result because the genotype of the pollen-producing plant is incompatible with stylar tissue (sporophytic incompatibility) or because the genotypes of individual pollen grains prevent successful growth of pollen tubes (gametophytic incompatibility: de Nettancourt 1977). The physiological mechanism of sporophytic self-incompatibility may be accompanied by floral morphological traits such as heterostyly that promote outbreeding by discouraging self-pollination (de Nettancourt 1977).

Dichogamy is one mechanism that discourages self-pollination (Lloyd and Webb 1986). Dichogamy is the temporal separation of male and female functions so that pollen presentation precedes stigma receptivity in protandrous individuals, whereas protogynous individuals function first as females and then as males (Bawa and Beach 1981, Lloyd and Webb 1986).

Plants may receive a mixture of pollen from various sources. The mixed mating model used for describing mating systems assumes that zygotes either result from self-fertilization or from random mating with other plants (Clegg 1980, Ritland 1983). Various models predict predominant inbreeding or outcrossing depending on the severity of inbreeding depression (Lloyd 1979, Lande and Schemske 1985, Schemske and Lande 1985), whereas others predict intermediate selfing rates (Lloyd 1979). However, high, low and intermediate outcrossing rates have all been observed

(Harding *et al.* 1974, Ellstrand *et al.* 1978, Schoen 1982) even among populations of a single species (Eckert and Barret 1992).

Part of this study will evaluate the breeding systems of monkshood and larkspur. I will determine the degree of self-incompatibility in these species and compare the results of self-pollination to known outcrosses and natural pollinations. The actual outcrossing rates of natural populations of monkshood and larkspur will be determined electrophoretically. The degree of protandry in the study species will also be examined.

2.1.2 Flowering phenology, competition for pollinator service and inadequate pollen receipt

From the perspective of animal-pollinated plants, pollinators are a resource and, therefore, inadequate pollination may limit reproductive success (Levin and Anderson 1970, Waser and Real 1979). Flowers offer nutritional resources to pollinators in various forms (nectar, pollen and floral oils) and the foraging of pollinators among conspecific plants, which these nutritional resources promote, makes them useful vectors for pollen transport.

The foraging behavior of pollinators can significantly affect the reproductive success of plants. From a plant's perspective an ideal pollinator would move only between conspecific plants and thus avoid wasting pollen and contaminating stigmas with pollen from other species. Indeed, pollinators do not move randomly among flower species as they forage, rather their behavior often incorporates some degree of constancy (Darwin 1876). Constancy is the tendency of individual pollinators to forage within a single species of plant in the presence of another suitable species. More specifically, it is the probability that a pollinator chooses the same species on successive visits (Levin and Anderson 1970), rather than visiting species entirely at

random. Waser (1986) reviewed the use of the term constancy, and suggested that it be used exclusively for pollinator behavior based on learning of floral mechanisms.

Several possible effects of pollinator inconstancy can compromise both male and female function in plants. Flowers that fail to attract pollinators due to the presence of other species suffer because of competition for pollinator preference (Free 1968, Rathcke 1983, Waser 1983). Competition through interspecific pollen transfer results from imperfect pollinator preference (Rathcke 1983, Waser 1983).

Interspecific pollen transfer reduces pollen flow between conspecifics, and receipt of heterospecific pollen on a stigma may reduce the ability of conspecific grains that reach the stigma to fertilize ovules (Campbell and Motten 1985).

Interspecifically transferred pollen may lead to loss of reproductive success (female function) in the receiving flowers (Waser and Fugate 1986, Galen and Gregory 1989). The converse, loss of pollen (male function) to heterospecific stigmas, may also result in lost reproductive opportunities. Campbell and Motten (1985) and Feinsinger and Tiebout (1991) found loss of pollen, rather than stigmatic interference between conspecific and heterospecific pollen, to be the more significant mechanism of interspecific interference. Waser (1978a) modelled the effects of interspecific pollen movement using computer simulations and found that the rapid exclusion of a plant species may result unless it is mitigated, perhaps by evolutionary divergence of flowering times. Rathcke (1983) also suggested that pollen limitation resulting from competitive interactions may lead to a separation of flowering times.

The opportunity for reproductive competition between plant species will depend on the overlap of flowering periods. Waser (1978b) measured seed set in sequentially flowering *Delphinium nelsonii* and *Ipomopsis aggregata* and found that the period of phenological overlap coincided with reduced reproductive success.

Gross and Werner (1983) similarly found that four sequentially flowering species of *Solidago* experienced lower seed set at periods of phenological overlap.

This study will determine whether opportunities for interspecific transfer are significant in populations of monkshood and larkspur whose flowering periods overlap and it will examine the effects of interspecific pollen transfer. There is the potential for competition for pollination between these two species because their flowering periods overlap significantly. Natural pollen deposition on stigmas will be surveyed and the proportion of heterospecific pollen determined.

2.1.3 Pollen receipt, multiple pollen donors and multiple paternity

Both the quality and quantity of pollen placed on receptive stigmas must be assessed when examining sexual reproduction in plants. From the perspective of female function, it is necessary to establish the paternity pool of a given plant (Levin 1988). As may be expected, a stigma generally receives most of its pollen from nearby or adjacent donors (Levin and Kerster 1974). The size of a paternity pool is a function of pollen dispersal and depends on factors such as plant density and pollinator behavior (Levin 1988). In particular, pollen from a given flower is often involved in pollinations many visits later in a succession of visits so that a single stigma can receive pollen from multiple origins (Thomson and Plowright 1980, Thomson and Thomson 1989).

Deposition of pollen of multiple origins on a single stigma means that 1) a particular ovule may be fertilized by one of several genetically different pollen grains and 2) the seeds within a given fruit may be sired by different pollen donors. Differential fertilization success among pollen grains on the same stigma may be realized by several means. Competition among the male gametophytes is a prezygotic mechanism in which faster growing pollen tubes are the successful fertilizers of

ovules (Lee 1984). Following fertilization, ovules that better enhance maternal fitness may be selectively matured (Bookman 1984, Temme 1986).

Janzen (1977) proposed that female flowers (or the female function in hermaphrodites) should sample an optimum number of the males in the population to balance the quality and number of potential fathers. Pollination by multiple donors allows for choice by the female with regard to seed paternity. A greater variety of pollen competing for the limited number of ovules may result in intense selection (Bertin 1982a, Stephenson and Bertin 1983, Snow 1986, Snow and Spira 1991). Multiple paternity may also result in kin selection and sibling rivalry among fertilized ovules within a single fruit (Kress 1981, Queller 1983).

Marshall and Ellstrand (1986) examined the implications of multiple paternity for sexual selection in *Raphanus sativus* and documented sexual selection operating primarily by male-male competition rather than by female choice. Pollen tubes growing through stylar tissue race with each other to fertilize available ovules; the fastest growing pollen tubes are from pollen grains which often produce the most vigorous offspring (Mulcahy 1974, Lee 1984, Snow 1986, Winsor *et al.* 1987). Ellstrand (1984) demonstrated that most fruits on a plant were multiply sired. Bertin (1990) demonstrated that larger pollen loads representing more pollen donors led to greater fruit and seed production and greater total seed mass in *Campsis radicans*.

2.1.4 Objectives

This study examines aspects of the reproductive biology of larkspur and monkshood. It will characterize intraspecific aspects of reproduction such as the degree of self-compatibility, the severity of inbreeding depression of seed number and size, natural levels of outcrossing and the effects of multiple paternity. It also determines to what extent female function in larkspur and monkshood is pollen limited and determines the prevalence and effects of interspecific pollen movement.

2.2 Methods

2.2.1 Description of *Aconitum delphinifolium* and *Delphinium glaucum* and study site

Monkshood, *Aconitum delphinifolium* DC (Ranunculaceae), is a perennial, hermaphroditic herb with short tubers, erect stems 3-7 dm tall, and bright blue, zygomorphic flowers (Moss 1983). Most plants produce one flowering stem (inflorescence) per plant with 1 to 10 flowers arranged in a short raceme; flowering occurs acropetally so that flowers at the base of the inflorescence bloom first. Each flower is protandrous, functioning first as a male and maturing female reproductive structures subsequently. The perfect flowers produce three to five carpels (generally three) and 30 to 40 stamens. Each carpel develops into a short dehiscent follicle containing up to 20 seeds. During the growing season the original tuber produces a collateral tuber from which the following year's ramet will be produced (Brink 1980). Brink (1980) documented the production of large tubers by large *A. columbianum* plants as well as the production of multiple tubers by single plants. I observed neither multiple stems produced by single tubers nor rhizomes connecting different individuals. Nor did I find any *A. delphinifolium* plants with multiple collateral tubers. However, large plants did tend to produce large collateral tubers.

Aconitum delphinifolium occurs in moist meadows throughout Alaska, Yukon and northern British Columbia (Porsild and Cody 1980) and reaches its southern limit in the Athabasca drainage of Alberta. Hultén (1968) included eastern Siberia in the range of *A. delphinifolium* subsp. *delphinifolium*. In Alberta, the range of monkshood is limited to a region in the west-central part of the province (Moss 1983).

Tall larkspur, *Delphinium glaucum* S. Wats. (Ranunculaceae), is a perennial hermaphroditic herb with erect stems 0.5-2 m tall, and purple zygomorphic flowers (Moss 1983). Racemes were 0.5-1 m tall at the study site and produced 10 to 40 flowers. Like monkshood, larkspur exhibits protandry and an acropetal flowering

sequence. The perfect flowers have three to five carpels (generally three) and 30 to 40 stamens. Each carpel forms a dehiscent follicle containing approximately 20 seeds on average. Multiple stems (up to 15) were observed emerging from a single elongated tuber, but generally larkspur individuals consisted of single flowering stems. Larkspur can apparently produce multiple stems year after year, as withered stems from the previous year emerged from the same tuber as current stems.

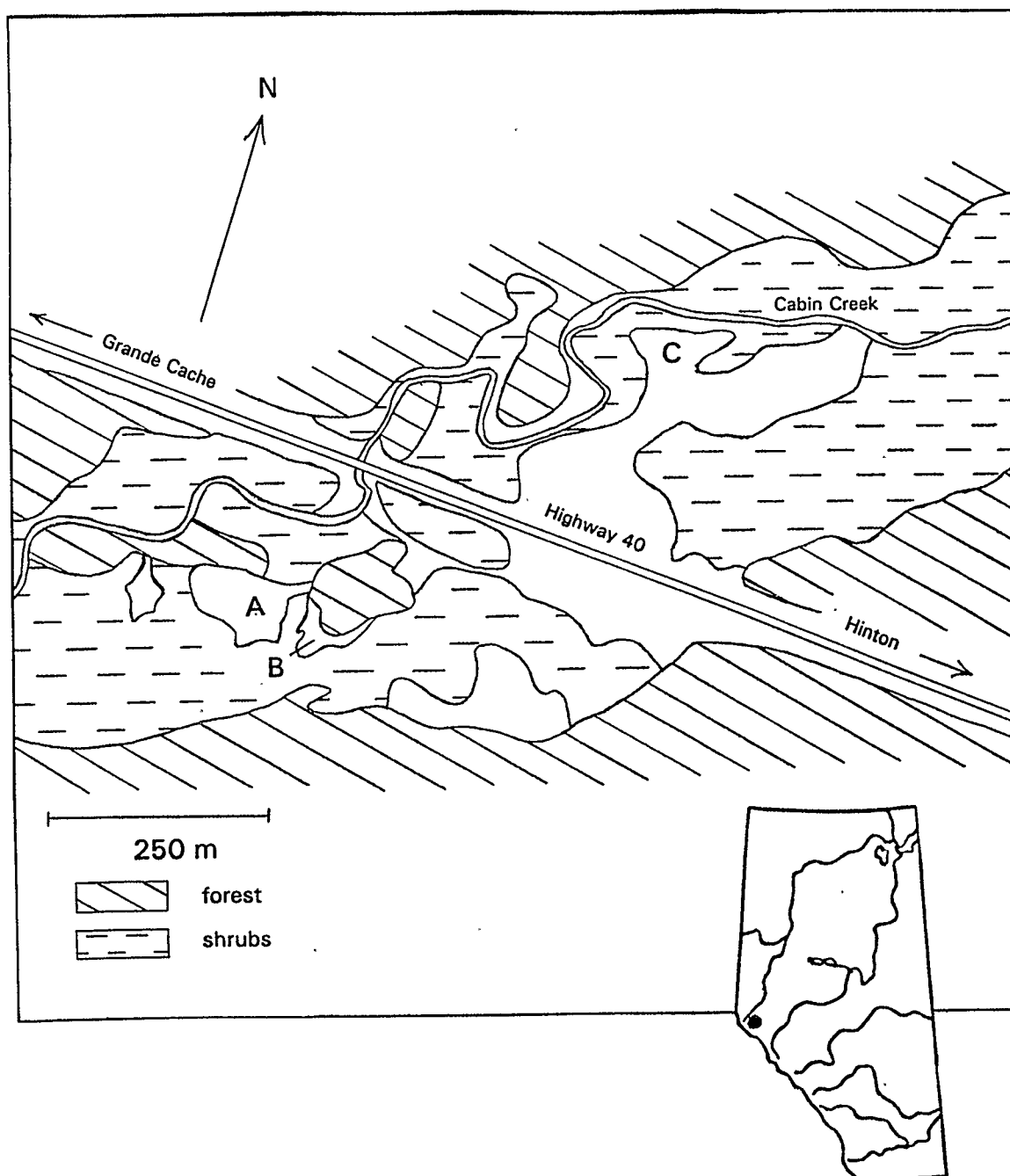
Tall larkspur occurs in Alaska, northern British Columbia and all of wooded Alberta (Hultén 1968, Porsild and Cody 1980, Moss 1983). Larkspur grows in moist woodlands and clearings (Porsild and Cody 1980).

I studied monkshood and larkspur primarily in a 0.5 ha meadow located adjacent to Cabin Creek between Hinton and Grande Cache, Alberta (53°46'N, 118°22'W, elevation 1525 m: see Figure 2.1, meadow A). Conditions at the site fit the preferred habitat descriptions for both monkshood and larkspur. Frequent rain and dew kept the soil moist beneath the ground cover of grasses, herbs and lichens. Larkspur begins flowering slightly earlier than monkshood at this site (see 2.3.2).

Only one other species bloomed in significant abundance during the flowering periods of *A. delphinifolium* and *D. glaucum*. Slender blue beard-tongue, *Penstemon procerus* Dougl. ex Benth. (Scrophulariaceae), began flowering before either study species, but had declined to only a fraction of its initial high flowering density by the time monkshood and larkspur flowered. The flowering density of *P. procerus* diminished to zero during the study. Grasses dominated the vegetation of the meadow.

An adjacent smaller (0.2 ha) meadow (Figure 2.1, meadow B), separated from the meadow A by a bog birch (*Betula glandulosa* Michx.) and willow (*Salix* spp.) thicket, contained only monkshood. The two meadows were separated by approximately 20 m. A third meadow (Figure 2.1, meadow C), located north of

Figure 2.1. Map of study site. Meadows A and B were the study sites in 1989. Meadow C was used in the 1990 examination of pollen and resource limitation of reproduction (see Chapter 3). Inset map indicates location of study site within Alberta.



Highway 40, was used in the 1990 examination of pollen and resource limitation of reproduction (see Chapter 3).

Two bumble-bee species, the long-tongued *Bombus flavifrons* Cresson, and the short-tongued *Bombus mixtus* Cresson, dominated the pollinator fauna of the study species. These bumble-bees foraged on larkspur and monkshood for the nectar contained in the nectar spurs of these flowers. The plants flower sequentially (with considerable overlap: see 2.3.2). Initially, *B. flavifrons* foraged on larkspur while *B. mixtus* visited *Penstemon procerus* because of its shorter tongue. *Bombus mixtus* switched to monkshood as it began flowering and *B. flavifrons* switched to monkshood as the density of larkspur declined. This transition by *B. flavifrons* provided opportunities for interspecific pollen transfer between larkspur and monkshood. The bumble bees typically visited flowers low on the inflorescence first and departed from flowers nearer the top.

2.2.2 Protandry

Individual monkshood and larkspur flowers function first as males and then as females; this protandry promotes outcrossing and reduces interference between male and female function (Lloyd and Webb 1986). To characterize the protandry of monkshood and larkspur, I monitored the development of flowers within inflorescences. The phase of each flower was recorded twice daily, at 900 h and 1700 h, from anthesis until petal loss which marked the end of female phase. During male phase, the stamens were upright and the stigmas were not visible among them. During female phase, the stamens had withered and bent outward and the styles elongated so that the stigmas occupied a central position. Female phase was characterized by splayed stigma lobes and the secretion of stigmatic fluid.

I monitored all flowers on 19 monkshood plants, which included from three to ten flowers during a nine-day period. Some flowers withered and failed to develop,

so the number of flowers on each plant for which the entire phenology was observed ($n=57$) ranged from one to six.

Given the large number of flowers in typical larkspur inflorescences, it was not feasible to observe the development of all flowers in an inflorescence, so only four flowers on each of 20 inflorescences were observed. The total flowering period of these 80 flowers, from the opening of the first bud to the end of the last female-phase flower spanned 21 days.

2.2.3 Flowering phenology

I characterized the flowering phenologies of larkspur and monkshood by measuring flower densities throughout the flowering period. Sixty 1-m² plots were randomly placed in meadow A along six parallel transects (10 plots/transect) spaced 10 m apart. The square plots were established using pin flags that remained unmoved for the duration of the study. At 48-h intervals I counted all inflorescences and all male- and female-phase flowers for each species within each plot.

2.2.4 Pollen received by stigmas

To quantify natural deposition of conspecific and heterospecific pollen on stigmas, I collected receptive stigmas from female flowers of both species. These female-phase flowers were almost finished flowering. Stigmas were removed from flowers of both species in the large meadows, A and C (Figure 2.1), and from monkshood in the small meadow B where larkspur did not occur. Stigmas from 38 larkspur and 55 monkshood plants were analyzed.

Stigmas and the pollen they bore were stored in 70% ethanol until they were processed by acetolysis (Faegri and Iverson 1989). This procedure digested the stigmatic tissue as well as the contents of the pollen grains but left the pollen exines intact. The acetolyzed pollen grains were permanently mounted in silicon oil for light

microscopy. Acetolysis facilitated viewing of the exine sculpturing of the pollen and permitted species identification of individual pollen grains.

Both monkshood and larkspur have *Caltha*-type pollen (Ridley 1988, Faegri and Iversen 1989), which is tricolpate, scabrate with fine regularly distributed spines and generally less than 25 μm in diameter (Ridley 1988, Faegri and Iversen 1989). Based on 60 grains of each species, size is not an effective criterion for differentiating monkshood (mean \pm SE diameter $19.8 \pm 0.11 \mu\text{m}$) and larkspur ($19.8 \pm 0.14 \mu\text{m}$). Fortunately, slight differences in the coarseness of the exine sculpturing allowed discrimination between the pollen of monkshood and larkspur. The entire pollen sample from each stigma was identified and counted under a light microscope (400X magnification).

2.2.5 Experimental pollinations

Several types of pollen transfer were possible for monkshood and larkspur. Natural self-pollination between male- and female-phase flowers on the same inflorescence (geitonogamous pollination) is possible if the pollinator visits a male-phase flower first. Outcrossing would occur when pollen from a male-phase flower on one inflorescence was deposited on a female-phase flower of a second inflorescence. Interspecific pollen transfer would occur if pollinators moved between plant species. I therefore conducted manual pollinations to examine the effects of self-pollination, outcrossing and interspecific pollen transfer on seed set in monkshood and larkspur.

To control the pollen received by stigmas, I manually pollinated emasculated flowers. Emasculation involved gently stripping anthers from their filaments with forceps while the flowers were yet buds. The altered inflorescences were then covered with nylon-mesh bags to exclude pollinators. When the emasculated flowers reached female phase and presented receptive stigmas, I applied the appropriate

pollination treatment. A freshly dehiscent anther was removed from the respective donor flower with forceps and brushed against the stigma of the recipient flower. Plants were then covered with mesh until the petals withered and fell off, when the mesh was removed and fruits were allowed to mature. Fruits were harvested two to three weeks later when they were about to dehisce and preserved in 70% ethanol for later examination of seed set.

Manual pollinations were conducted during three periods which coincided with differing behaviors in the two pollinator species. Series 1 occurred early in the season when larkspur flowering density exceeded monkshood density; during series 2 monkshood density exceeded that of larkspur and was increasing rapidly; and series 3 occurred during greatest monkshood density. Pollinators switched plants as plant densities changed, creating the opportunity for interspecific pollen transfer. However, I was unable to execute the three series of pollinations at exactly the times of minimum and maximum phenological overlap. Although the pollinations were not conducted at the ideal times described they still occurred under different plant densities.

Only larkspur flowers were pollinated during series 1. Four flowers were emasculated on each of 30 inflorescences: two flowers received geitonogamous self pollen and the other two flowers received outcrossed pollen from other inflorescences. Self pollinations involved receipt of pollen from two or, if possible, three flowers on the same inflorescence, whereas all outcrosses involved three pollen donors per recipient flower. Hand-pollinated flowers received their designated treatment on two separate occasions to ensure pollination during stigma receptivity. I randomized pollination treatments among flowers within an inflorescence to ameliorate any effects of flower position on seed set.

Series 2 pollinations involved both plant species. Three flowers were emasculated on all experimental plants: two flowers received mixtures of conspecific and heterospecific pollen and the third received outcross pollen. The pollen mixtures involved sequential applications of conspecific and heterospecific pollen; one flower received conspecific pollen followed by heterospecific pollen and the second flower was pollinated in reverse order. In addition to meadow A, where both monkshood and larkspur were treated, I pollinated monkshood in meadow B. These monkshood plants were potentially subject to different reproductive conditions given the absence of larkspur plants. A total of 90 experimental plants was involved: 30 of each species in meadow A and 30 monkshood plants in meadow B.

Series 3 pollinations involved only monkshood in both meadows A and B. Two flowers on each plant received outcross pollen. No self pollination or interspecific crosses were performed in this series. A separate examination of the effects of self-pollination and outcrossing in monkshood was carried out in the summer of 1988. The standard emasculation and exclusion procedure was used. On each of 30 inflorescences one flower was geitonogamously pollinated and two flowers were outcrossed.

Interspecific pollinations were repeated during the 1990 field season during greatest monkshood density. Thirty monkshood and larkspur plants were selected and two flowers on each plant were emasculated and pollinators were excluded. One flower received only heterospecific pollen; whereas the other received heterospecific pollen followed by conspecific pollen. A third unmanipulated flower was chosen a priori to monitor open pollination. This open-pollinated flower was selected low on the inflorescence so that it would not be enclosed in the mesh bag.

During all pollination series, I monitored fruit and seed set by plants that had experienced ambient pollination. I identified the fruits to be collected prior to fruit

set. The number of fruits collected from unmanipulated plants in each round was identical to the number of hand-pollinated flowers on the experimental plants. Additionally, I collected data on plant size for each experimental and control plant to be used as covariates in the analysis. These covariates were inflorescence size and relative flower position (measured acropetally).

2.2.6 Multiple paternity

During 1990 I examined the potential effects of multiple paternity for seed production by monkshood and larkspur using manual pollinations. I selected 30 plants of each species in meadow A and followed the usual emasculation and bagging procedure for three flowers on each plant. Following manual pollination, plants were bagged again until petals dropped and fruit development was underway.

The three flowers within a plant were randomly assigned to receive pollen from one, three or five donors. All pollinations involved pollen from other plants. A freshly dehiscent anther was removed from a donor flower and used for a single pollination. The three experimental flowers on a plant received pollen from a total of nine donor plants. In pollinating flowers that received three or five pollinations I applied the pollen from each male sequentially and brushed it around on the stigma to mix the donor pollen.

2.2.7 Harvest and examination of fruit

All fruits were stored in 70% ethanol in 1.5 ml microcentrifuge tubes until they could be examined further. Fruits were dissected under a stereo dissecting scope (60X magnification). The follicles were easily split open with forceps to reveal the contents. The number of seeds, unfertilized ovules and aborted seeds were determined for each fruit. A well-developed seed was round and firm within a loose, wrinkled seed coat. I defined aborted seeds as those that had a partially filled seed or

were blackened and mushy. The seeds produced by each flower were then dried overnight at 60°C and weighed to determine dry mass.

2.2.8 Statistical analysis

I used analyses of covariance to assess sources of variation in the durations of male- and female-phase in flowers, deposition of pollen on stigmas, the proportion of ovules setting seed (seed set) and seed mass. Analyses included main effects, covariates and interactions, with backward elimination of insignificant terms. Different covariates were included in different analyses and these are indicated in summary tables for each. Typical covariates were inflorescence size and relative flower position. When covariates affected treatment groups homogeneously I report means as though all plants were measured at the mean value of the covariate. However, partial regression coefficients are reported when covariates affected treatment groups heterogeneously. After detecting significant main treatment effects I performed multiple comparison tests between the different levels for the particular treatment.

The analysis of male- and female-phase durations in the examination of protandry involved a repeated-measures design with one between-flower factor (plant, which was treated as a random factor) and one within flower factor (sexual phase).

In analyzing seed set, I treated seed set by individual ovules as a binomial process. The proportion of successes (p) was logistically transformed ($\ln[p/1-p]$: Neter *et al.* 1985) prior to analysis. I was interested only in ovules that set seed, so cases where $p=0$ were deleted. If $p=1$ then the datum was modified as $p=1-1/2n$, where n was the number of ovules, to avoid division by zero during transformation. To correct for the unequal variances associated with a binomial process, the analyses used weighted least squares, with each observation being weighted by the inverse of the variance for a logit based on n ovules ($np[1-p]$). In describing my results I

present back-transformed means and standard errors. Back-transformation results in asymmetric standard errors (SE); these are presented as lower SE (LSE) and upper SE (USE).

Seed mass data were normalized using a square-root transformation prior to analysis. Analyses of seed mass also included the number of seeds and ovules per flower as additional covariates. Back-transformation yielded asymmetric standard errors and these are presented as lower SE (LSE) and upper SE (USE) as well.

2.2.9 Electrophoretic examination of outcrossing

I used cellulose-acetate electrophoresis of isozymes to determine the outcrossing rates for monkshood and larkspur. Flower buds and developing follicles were collected from plants of each species. Following transport on ice, the plant tissues were stored in an ultracold freezer (-70°C) until processing. Flower bud tissue and nine seeds from each family were used so that progeny genotypes could be compared to maternal genotypes.

Monkshood and larkspur contain many secondary plant compounds which render isozymes useless upon maceration of the plant tissue. Therefore, the flower buds and seeds were ground up in an extraction buffer designed to neutralize these substances (Wendel and Weeden 1989). The electrophoretic apparatus used a continuous buffer system with a CAM buffer similar to the CAEA buffer of Hebert and Beaton (1989) as both gel and electrode buffers. (The CAM buffer replaces N-(3-aminopropyl)-diethylamine with N-(3-aminopropyl)-morpholine). Current was applied across each cellulose-acetate plate for 80 min (6 mA per plate) under refrigerated conditions (4°C).

An initial survey of isozymes in monkshood and larkspur found monkshood to be polymorphic for leucine aminopeptidase (LAP), isocitrate dehydrogenase (IDH) and phosphoglucomutase (PGM). Larkspur was polymorphic for LAP, IDH and

phosphoglucose isomerase (PGI). I stained the gels for these isozymes following the application of current and scored them immediately.

Analysis of outcrossing rates was performed using software based on the multilocus estimation methodology of Ritland and Jain (1981) which also provided boot-strapped standard errors associated with the estimated outcrossing rates.

2.3 Results

2.3.1 Protandry

The male and female phases of individual monkshood and larkspur flowers differed significantly in duration. In monkshood, male phase lasted for an average (\pm SE) of 111.2 ± 2.14 h ($n=57$) whereas female phase lasted only 50.3 ± 2.27 h ($n=57$). This difference between duration of male and female phases was significant but the relationship between them depended on plant identity (Table 2.1a).

Male phase for single larkspur flowers lasted for an average of 137.4 ± 2.79 h ($n=76$) while the flowers were in female phase for an average of only 36.8 ± 1.69 h ($n=76$). The difference in the average time that a larkspur flower was in male or female phase was highly significant but depended on plant identity (Table 2.1b).

2.3.2 Flowering phenology

During both 1989 and 1990 the flowering density (flowers/m²) of monkshood in meadow A at a given time typically surpassed that of larkspur (Figure 2.2). Densities of monkshood in the two years were comparable, but the observed density of larkspur was much lower the second year.

Within each species, peak density of male-phase flowers exceeded peak density of female-phase flowers (Figure 2.3). As expected for protandrous plants, the peak density for female-phase flowers followed that of male-phase flowers, but only

Table 2.1a. Analysis of protandry in monkshood.

Effects	test statistic
Between flower factor	
plant	$F_{19,16} = 0.46$
Within flower factors	
sexual phase	$F_{1,21} = 123.09^{***}$
phase x plant	$F_{19,38} = 6.07^{***}$

*** $P < 0.001$

Table 2.1b. Analysis of protandry in larkspur.

Effects	test statistic
Between flower factor	
plant	$F_{18,15} = 0.58$
Within flower factors	
sexual phase	$F_{1,18} = 246.60^{***}$
phase x plant	$F_{18,57} = 7.43^{***}$

*** $P < 0.001$

Figure 2.2. Total flower densities in meadow A during 1989 and 1990. The numbers above the abscissa for 1989 indicate the date of pollination series 1, 2 and 3. AD indicates curves describing monkshood densities and DG indicates those for larkspur.

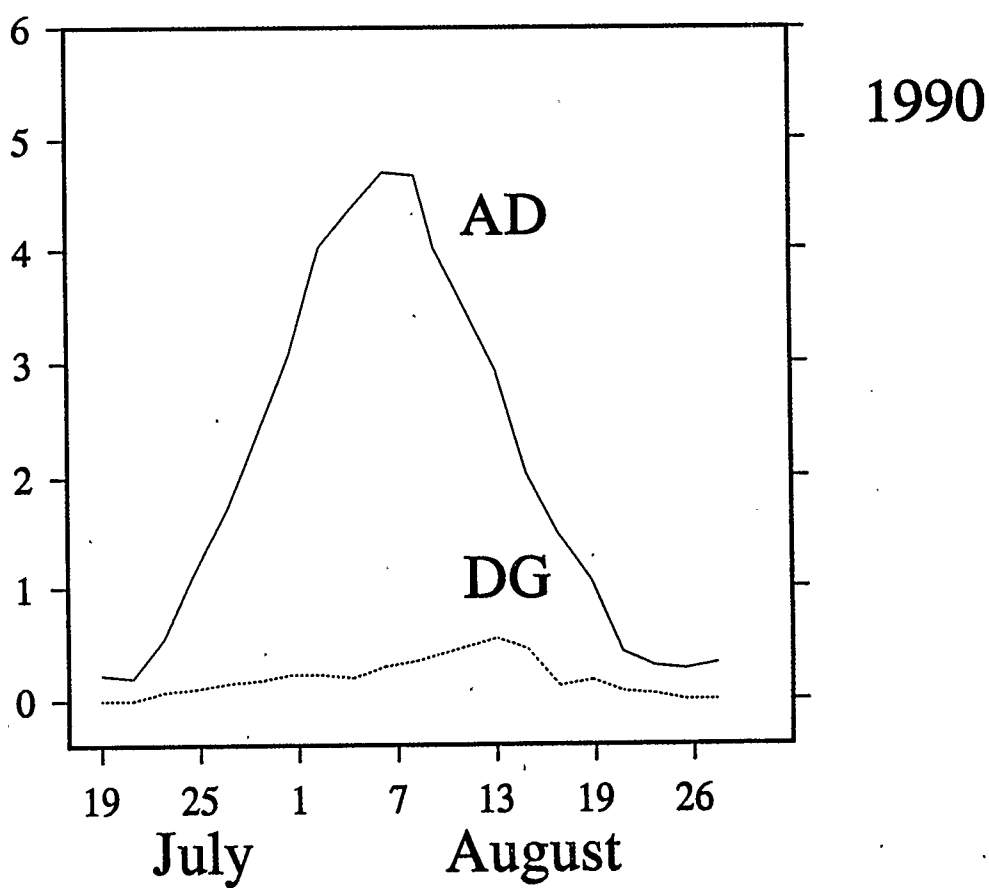
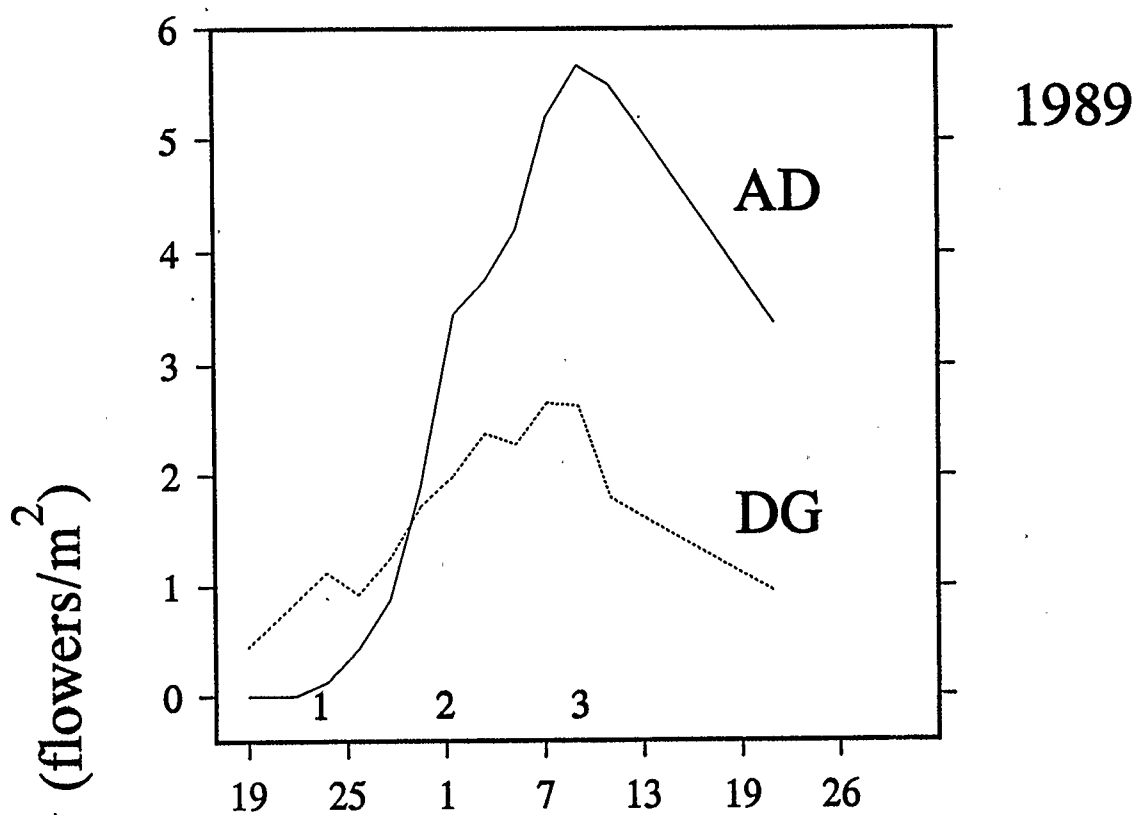
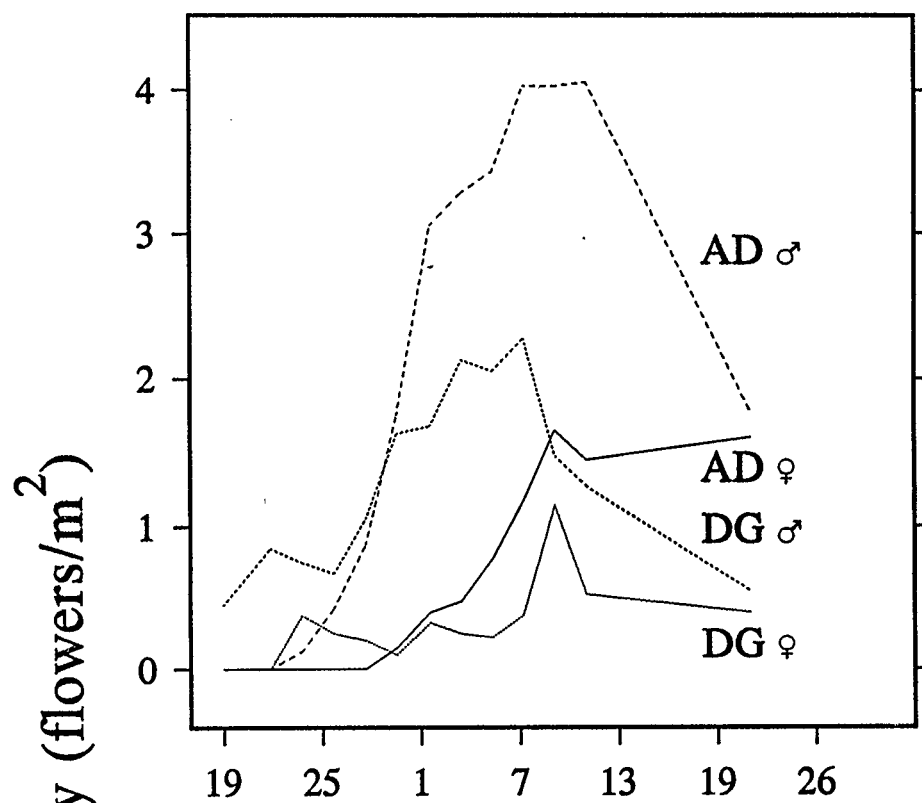
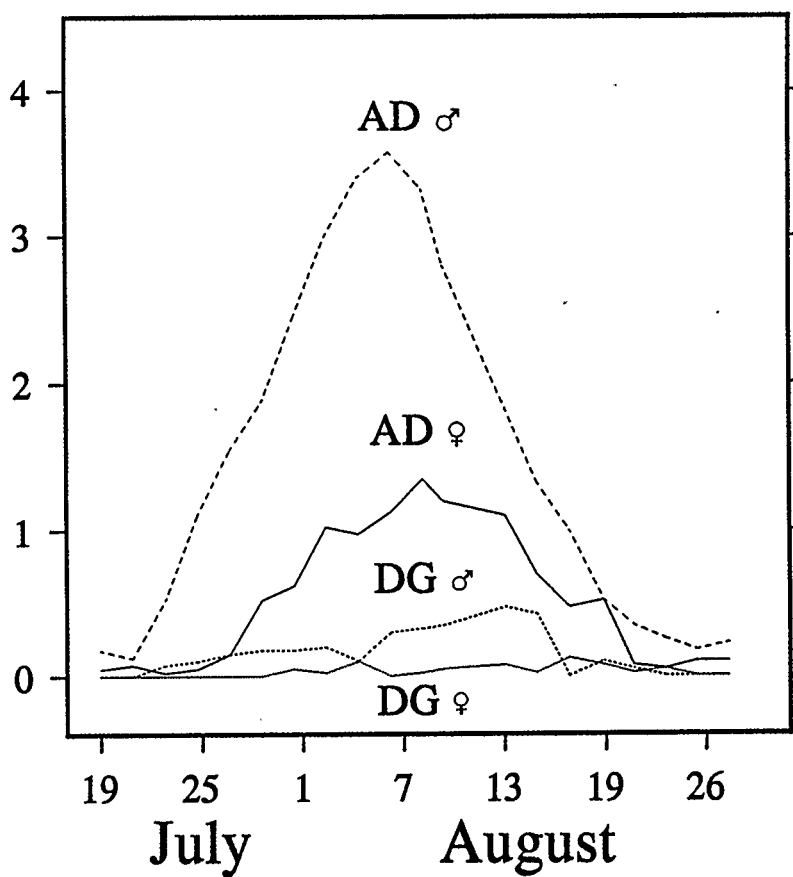


Figure 2.3. Total flower densities by sexual phase in meadow A during 1989 and 1990. AD indicates curves describing monkshood densities and DG indicates those for larkspur.

1989



1990



briefly. Both male- and female-phase flowers may be present on an inflorescence simultaneously.

The disparity between monkshood and larkspur densities was even more exaggerated when viewed as inflorescence rather than flower densities (Figure 2.4), because larkspur produces more flowers per inflorescence, with more flowers open simultaneously than monkshood. Inflorescence density more accurately describes the appearance of the meadow. The net effect is that for most of the flowering season there were more monkshood flowers available in the meadow and these flowers were distributed over more inflorescences.

Monkshood inflorescence sizes differed between meadow locations and pollination series (Table 2.2). Mean (\pm SE) inflorescence size of plants in meadow A was 5.2 ± 0.21 flowers ($n=60$) and those in meadow B was 4.5 ± 0.20 flowers ($n=60$). Open-pollinated monkshood inflorescences used in the earlier pollination series bore a mean (\pm SE) of 5.3 ± 0.23 flowers ($n=60$) whereas those used in series 3 had only 4.5 ± 0.18 flowers ($n=60$).

Larkspur inflorescences in meadow A bore 23.1 ± 0.85 flowers ($n=101$) in series 1 and 19.1 ± 0.78 flowers ($n=72$) in series 2. The decline in mean inflorescence size between the two pollination series was significant (2-sample t -test, $t_{171} = 3.29$, $P < 0.01$)

2.3.3 Pollen received by stigmas

The two species differed significantly in the amount of pollen found on randomly collected stigmas (Table 2.3). Monkshood stigmas received an average (\pm SE) of 74 ± 9.0 pollen grains ($n=55$) whereas larkspur stigmas received 16 ± 11.9 grains ($n=38$). Most of the pollen found on any stigma was conspecific. Monkshood stigmas received 72 ± 8.7 (97%) conspecific grains of pollen whereas larkspur had 14 ± 11.6 (88%) conspecific grains. The number of conspecific grains on stigmas

Figure 2.4. Densities of flowering inflorescences in meadow A during 1989 and 1990. AD indicates curves describing monkshood densities and DG indicates those for larkspur.

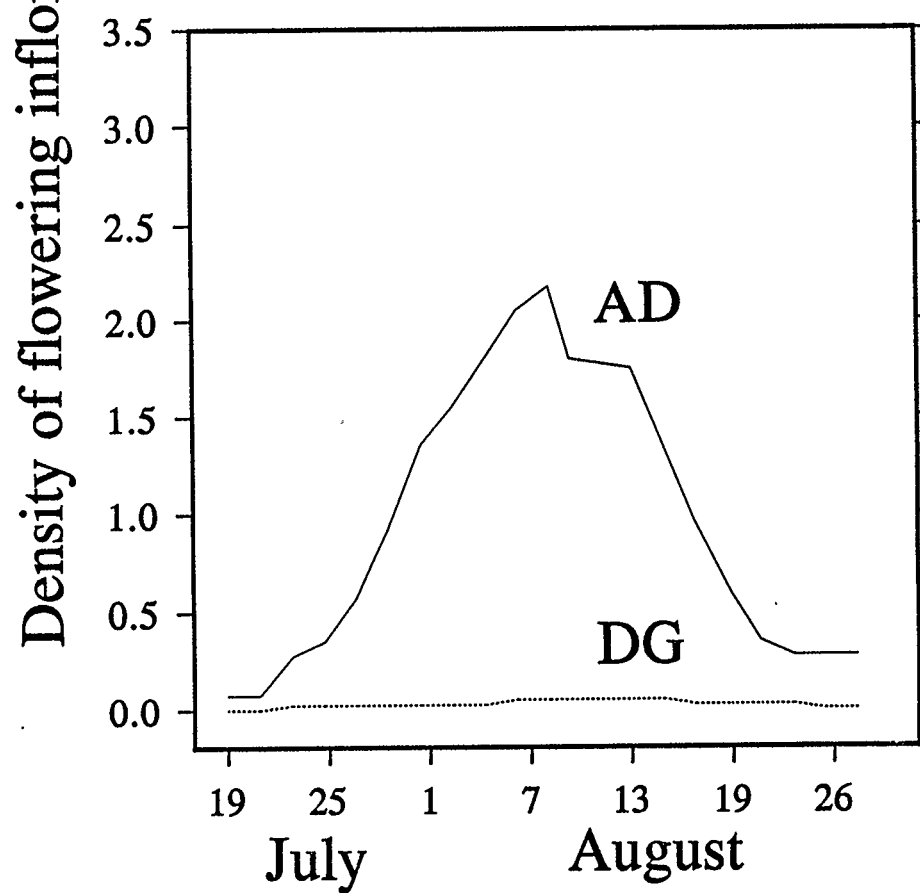
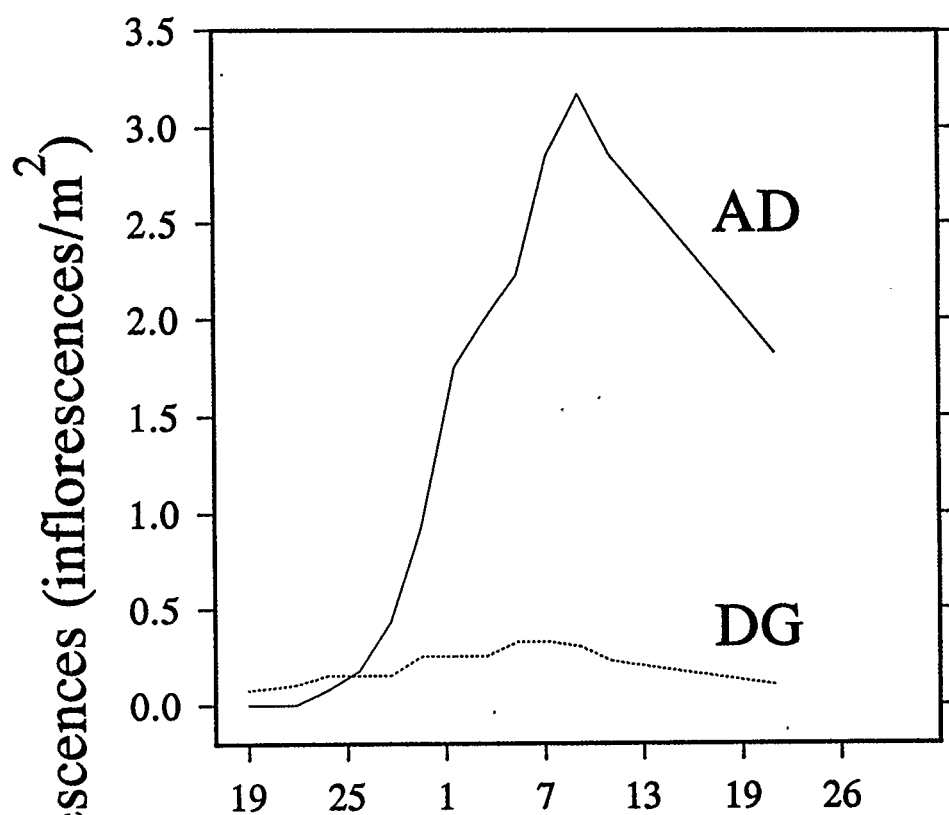


Table 2.2. Analysis of inflorescence sizes of open-pollinated monkshood in meadows A and B and pollination series 2 and 3.

Effects	test statistic
meadow location	$F_{1,116} = 6.42^*$
pollination series	$F_{1,116} = 7.67^{**}$
meadow x series	$F_{1,116} = 0.42$

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

Table 2.3. Analysis of number of pollen grains deposited on stigmas.

Effects ¹	total	conspecific	heterospecific
species	$F_{1,91} = 15.72^{***}$	$F_{1,91} = 17.94^{***}$	$F_{1,91} = 0.01$
male flowers	-	-	$F_{1,90} = 5.43^*$

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

¹Inflorescence size and relative flower position were not significant as covariates and were excluded; the number of male flowers per inflorescence was included only in the analysis of heterospecific pollen deposition.

differed significantly depending on species (Table 2.3). The number of heterospecific grains found on each stigma did not differ between species but did vary positively with the number of male-phase flowers on the plant from which the stigmas were taken ($b \pm s_b = 0.31 \pm 0.133$, $t_{92} = 2.33$, $P < 0.05$).

2.3.4 Seed set by open-pollinated plants

2.3.4.1 Larkspur

Seed set in open-pollinated larkspur did not differ for series 1 and series 2 (Table 2.4: see Fig. 2.2a for timing of these samples relative to the flowering phenology). The probability of seed set for ovules was 0.51 (LSE=0.484, USE=0.528, $n=101$) in series 1 and 0.47 (LSE=0.445, USE=0.502, $n=72$) in series 2.

However, seed mass differed significantly for larkspur from series 1 and series 2 (Table 2.4). Mean seed mass in series 1 was 0.46 mg (LSE=0.452, USE=0.475, $n=100$) and 0.18 mg (LSE=0.170, USE=0.187, $n=72$) in series 2. I allowed similar lengths of time for fruits in each series to mature and harvested them when they were large enough that it would be possible to determine the status of the seeds within. Unfortunately, I was not stringent in maintaining identical fruit maturation times and even slight differences between series may have impacted directly on seed mass. Therefore I will not attempt to interpret the effects of time of pollination on seed mass nor of any interactions involving this factor. Seed mass increased with both increasing inflorescence size ($b \pm s_b = 0.0023 \pm 0.00085$, $t_{168} = 2.69$, $P < 0.01$) and relative flower position ($b \pm s_b = 0.082 \pm 0.0378$, $t_{168} = 2.16$, $P < 0.05$).

Table 2.4. Analysis of seed set and seed mass for open-pollinated larkspur.

Effects ¹	seed set	seed mass
pollination series	$F_{1,171} = 0.81$	$F_{1,168} = 373.23^{***}$
inflorescence size	-	$F_{1,168} = 7.21^{**}$
relative flower position	-	$F_{1,168} = 4.64^*$

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

¹Inflorescence size and relative flower position were not significant as covariates for seed set and were excluded.

2.3.4.2 Monkshood

Seed set in open-pollinated monkshood varied significantly depending on meadow location (Table 2.5). In meadow B, where larkspur was absent, monkshood ovules set seed with a probability of 0.62 (LSE=0.599, USE=0.639, $n=118$). The chance of seed set was only 0.49 (LSE=0.474, USE=0.517, $n=105$) in meadow A. Seed set was unaffected by pollination series. There was no significant interaction between these two main treatment effects.

Seed mass in open-pollinated monkshood depended significantly on the interaction between meadow location, pollination series and relative flower position (Table 2.5). The time effect will be ignored (see 2.3.4.1). The interaction between relative flower position and meadow location resulted because seed mass declined significantly with position in meadow B ($b \pm s_b = -0.13 \pm 0.026$, $t_{210} = -5.03$, $P < 0.001$) but did not vary with position for the plants in meadow A ($b \pm s_b = -0.0053 \pm 0.02890$, $t_{210} = -0.18$, $P > 0.85$). Seed mass also increased with the number of ovules per flower ($b \pm s_b = 0.0011 \pm 0.00044$, $t_{210} = 2.52$, $P < 0.05$).

2.3.5 Effects of the quality of pollen received

2.3.5.1 Self-compatibility and breeding system

2.3.5.1.1 Larkspur

Manual outcrosses and self-pollinations (geitonogamous pollinations) in series 1 indicated that larkspur is self-compatible but may experience inbreeding depression. Self-pollinated flowers set a similar proportion of seeds as flowers that were outcrossed (Table 2.6). Ovules set seed with a probability of 0.39 (LSE=0.361, USE= 0.426, $n=43$) for outcrossed flowers and 0.31 (LSE=0.278, USE=0.337, $n=42$) for self-pollinated flowers.

Seed mass differed significantly between pollination treatments (Table 2.6). The average seed produced by self fertilization had a mass of 0.40 mg (LSE=0.383,

Table 2.5. Analysis of seed set and seed mass for open-pollinated monkshood.

Effects ¹	seed set	seed mass
meadow location	$F_{1,219} = 17.18^{***}$	$F_{1,210} = 8.35^{**}$
pollination series	$F_{1,219} = 3.45$	$F_{1,210} = 10.29^{**}$
meadow x series	$F_{1,219} = 0.25$	$F_{1,210} = 1.25$
number of ovules	-	$F_{1,210} = 6.36^*$
relative flower position	-	$F_{1,210} = 12.01^{***}$
position x series	-	$F_{1,210} = 4.64^*$
position x meadow	-	$F_{1,210} = 11.04^{***}$
position x meadow x series	-	$F_{1,210} = 3.95^*$

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

¹Inflorescence size and relative flower position were not significant covariates for seed set and were excluded from the analysis. Relative flower position and number of ovules per flower were retained as covariates for seed mass but inflorescence size and seed number per flower were excluded.

Table 2.6. Analysis of the effects of outcrossing and self-pollination on seed set and seed mass in larkspur.

Effects	seed set	seed mass
Between plant factor		
plant	$F_{23,22} = 3.26^{**}$	$F_{23,21} = 11.14^{***}$
Within plant factors		
pollination treatment	$F_{1,43} = 0.89$	$F_{1,22} = 16.47^{***}$
plant x treatment	$F_{22,38} = 1.55$	$F_{21,37} = 1.34$

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

USE=0.420, $n=41$), whereas those resulting from outcrossing had an average mass of 0.46 mg (LSE=0.443, USE=0.483, $n=42$). Average mass per seed decreased as the number of ovules per flower increased ($b \pm s_b = -0.0031 \pm 0.00111$, $t_{80} = -2.79$, $P < 0.01$).

Even though larkspur is self-compatible, most open-pollinated seeds are outcrossed as indicated by the estimated multilocus outcrossing rate of 0.946 (SE=0.134).

2.3.5.1.2 Monkshood

There was no difference in seed set for selfed and outcrossed monkshood flowers ($F_{1,46} = 0.25$). The probability of seed set was 0.41 (LSE=0.364, USE=0.459, $n=31$) for outcrossed ovules and 0.45 (LSE=0.386, USE= 0.519, $n=17$) for self-fertilized ovules. Seed mass was not analyzed for selfed and outcrossed monkshood because the experiment was conducted in 1988 before I became interested in pollination effects on seed size.

As with larkspur, most ovules subject to open-pollination are outcrossed as indicated by the estimated multilocus outcrossing rate of 0.945 (SE=0.087).

2.3.5.2 Comparison of outcrossed and open-pollinated flowers

2.3.5.2.1 Larkspur

Analysis of manually outcrossed and open-pollinated flowers from series 1 and series 2 revealed that pollination treatment significantly affected the probability of seed set (Table 2.7a). The probability of seed set was 0.48 (LSE=0.466, USE=0.502, $n=173$) for open-pollinated plants and 0.39 (LSE=0.357, USE=0.423, $n=64$) for outcrossed plants. The results of the pollination treatments did not differ from series 1 to series 2.

Table 2.7a. Analysis of the effects of outcrossing and open-pollination on seed set and seed mass in larkspur.

Effects ¹	seed set	seed mass
pollination treatment	$F_{1,229} = 7.85^{**}$	$F_{1,226} = 3.17$
pollination series	$F_{1,229} = 0.29$	$F_{1,226} = 44.65^{***}$
treatment x series	$F_{1,229} = 2.41$	$F_{1,226} = 0.49$
relative flower position	$F_{1,229} = 0.24$	-
position x treat x series	$F_{3,229} = 3.21^*$	-
inflorescence size	-	$F_{1,226} = 0.20$
number of ovules	-	$F_{1,226} = 0.47$
inflorescence x treatment	-	$F_{1,226} = 4.39^*$
ovules x series	-	$F_{1,226} = 5.98^*$

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

¹Only relative flower position was retained as a covariate for seed set.

Inflorescence size and the number of ovules per flower were retained as covariates for seed mass; relative flower position and seed number per flower were excluded.

Table 2.7b. Influence of relative flower position, pollination treatment and pollination series on seed set in larkspur.

		$b \pm s_b$
open-pollination	series 1	$-1.38 \pm 0.558^*$
	series 2	0.34 ± 0.604
outcross	series 1	1.53 ± 0.843
	series 2	0.27 ± 0.989

* $P < 0.05$

Relative flower position also affected seed set, but its influence depended on pollination treatment and time of pollination (Table 2.7b). For open-pollinated plants the relationship between seed set probability and relative flower position was negative in series 1, but there was not a significant relationship in series 2. For manually outcrossed plants the relationship did not differ from zero in either series; relative flower position did not affect seed set in these flowers.

Open-pollination and manual outcrossing produced seeds of equal mass. Pollination series was the only main treatment to significantly affect seed mass in larkspur (Table 2.7a). Again, the effects of date (series) of pollination on seed mass will not be considered (see 2.3.4.1). The interaction between inflorescence size and pollination treatment resulted because seed mass increased with inflorescence size for open-pollinated plants ($b \pm s_b = 0.0025 \pm 0.00086$, $t_{226} = 3.24$, $P < 0.01$), but not for manually outcrossed plants ($b \pm s_b = -0.0016 \pm 0.00180$, $t_{226} = -0.90$, $P > 0.35$). In open-pollinated plants, larger inflorescences produced heavier seeds. The interaction between ovule number per flower and pollination series will not be considered because of the problems with date of pollination.

2.3.5.2.2 Monkshood

A three-factor analysis of the monkshood pollinations in series 2 and series 3 revealed that pollination treatment, meadow location and pollination series all significantly affected the probability of seed set (Table 2.8). There was also a significant interaction between pollination treatment and series which resulted because the probability of seed set for hand-outcrossed monkshood in series 2 was lower than that of open-pollinated plants, but seed set was identical under the two treatments in series 3 (Table 2.9). Probability of seed set differed between meadow A and meadow B (Table 2.8). Probability of seed set in meadow A was 0.48 (LSE=0.457, USE=0.494, $n=176$) but was 0.58 (LSE=0.564, USE=0.600, $n=193$) in meadow

Table 2.8. Analysis of the effects of outcrossing and open-pollination on seed set and seed mass in monkshood.

Effects	seed set ¹	seed mass ²
pollination treatment	$F_{1,360} = 4.06^*$	$F_{1,350} = 3.88^*$
meadow location	$F_{1,360} = 16.60^{***}$	$F_{1,350} = 1.84$
treatment x meadow	$F_{1,360} = 0.65$	$F_{1,350} = 6.29^*$
pollination series	$F_{1,360} = 21.45^{***}$	$F_{1,350} = 23.12^{***}$
treatment x series	$F_{1,360} = 3.92^*$	$F_{1,350} = 7.51^{**}$
meadow x series	$F_{1,360} = 0.12$	$F_{1,350} = 1.47$
treat x meadow x series	$F_{1,360} = 0.00$	$F_{1,350} = 2.06$
relative flower position	$F_{1,360} = 5.51^*$	$F_{1,350} = 28.35^{***}$
number of ovules	-	$F_{1,350} = 1.65$
ovules x treatment	-	$F_{1,350} = 5.27^*$
position x meadow	-	$F_{1,350} = 8.23^{**}$
position x series	-	$F_{1,350} = 4.30^*$
position x meadow x series	-	$F_{1,350} = 8.39^{**}$

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

¹Relative flower position was the only covariate included for seed set; inflorescence size was not significant.

²Relative flower position and ovule number per flower were included as covariates for seed mass; inflorescence size and seed number per flower were excluded.

Table 2.9. Influence of pollination treatment and pollination series on seed set probability in monkshood. Tabled values are mean (\pm SE) seed set probabilities under treatment conditions. Lower SE (LSE) and upper SE (USE) are given separately following back-transformation.

	series 2	series 3
open-pollinated	0.52 0.449 - 0.540 $n = 122$	0.59 0.569 - 0.613 $n = 101$
hand-outcrossed	0.42 0.380 - 0.451 $n = 40$	0.59 0.568 - 0.611 $n = 106$

B where there were no larkspur present. Probability of seed set decreased with relative flower position within the inflorescence ($b \pm s_b = -0.55 \pm 0.2328$, $t_{360} = -2.35$, $P < 0.05$).

A significant interaction between pollination treatment and meadow location affected seed mass (Table 2.8). Seed mass was similar for open-pollinated plants in both meadows A and B. However, outcrossed plants in meadow B produced smaller seeds than those in meadow A (Table 2.10).

The interaction between relative flower position, meadow location and pollination series (Table 2.8) will not be considered. However, the interaction between relative flower position and meadow location resulted because seed mass in meadow B declined significantly with increasing relative flower position ($b \pm s_b = -0.11 \pm 0.019$, $t_{350} = -6.03$, $P < 0.001$), whereas there was no effect in meadow A ($b \pm s_b = -0.037 \pm 0.0199$, $t_{350} = -1.84$, $P > 0.05$). The interaction between pollination treatment and number of ovules per flower was caused by the significant increase in seed mass with ovule number for open-pollinated flowers ($b \pm s_b = 0.0011 \pm 0.00039$, $t_{350} = 2.84$, $P < 0.01$), but not for outcrossed flowers ($b \pm s_b = -0.00028 \pm 0.000482$, $t_{350} = -0.59$, $P > 0.55$).

2.3.5.3 Effects of multiple paternity

The number of pollen donors did not significantly affect seed set or seed mass in larkspur (Table 2.11a). The probability of seed set with one pollen donor was 0.39 (LSE=0.280, USE=0.509, $n=28$), 0.40 with three donors (LSE=0.285, USE=0.531, $n=28$) and 0.37 with five donors (LSE=0.266, USE=0.478, $n=29$). Variation in seed set and seed mass was due to individual effects of different plants (Table 2.11a).

For monkshood the probability that an ovule would set seed was 0.33 (LSE=0.195, USE=0.499, $n=22$) for ovules pollinated by one donor, 0.47

Table 2.10. Influence of pollination treatment, meadow location and pollination series on seed mass in monkshood. Tabled values are mean (\pm SE) seed masses (mg) under treatment conditions. Lower SE (LSE) and upper SE (USE) are given separately following back-transformation.

	open-pollinated	hand-outcrossed
meadow A	0.11 0.106 - 0.113 $n = 104$	0.11 0.109 - 0.118 $n = 71$
meadow B	0.11 0.104 - 0.110 $n = 115$	0.09 0.055 - 0.138 $n = 74$
series 2	0.12 0.113 - 0.120 $n = 119$	0.12 0.115 - 0.127 $n = 39$
series 3	0.10 0.097 - 0.104 $n = 100$	0.09 0.082 - 0.088 $n = 106$

Table 2.11a. Analysis of the effects of multiple pollen donors on seed set and seed mass in larkspur.

Effects	seed set	seed mass
plant	$F_{28,54} = 2.75^{***}$	$F_{24,34} = 3.50^{***}$
number of donors	$F_{2,54} = 0.26$	$F_{2,34} = 0.64$

*** $P < 0.001$

Table 2.11b. Analysis of the effects of multiple pollen donors on seed set and seed mass in monkshood.

Effects	seed set	seed mass
plant	$F_{25,43} = 1.20$	$F_{22,32} = 0.90$
number of donors	$F_{2,43} = 3.06$	$F_{2,32} = 1.80$

(LSE=0.317, USE=0.633, $n=24$) for three donors and 0.43 (LSE=0.290, USE=0.582, $n=25$) for five donors. The effect of the number of pollen donors on seed set probability was not significant (Table 2.11b). Seed mass did not vary with the number of pollen donors and individual plant identity also did not influence seed set and seed mass (Table 2.11b).

2.3.5.4 Effects of interspecific pollen transfer

During 1989, I interspecifically pollinated larkspur and monkshood only during the second of the three series of pollinations. For the interspecific pollinations of 1990, the analyses include flowers receiving the pollen mixture, the heterospecifically-pollinated flower and the open-pollinated flower.

2.3.5.4.1 Larkspur

Seed set by larkspur did not differ significantly between the two mixed pollination treatments in 1989 (Table 2.12). The mean probability of seed set for flowers receiving conspecific pollen followed by heterospecific pollen was 0.39 (LSE=0.331, USE=0.457, $n=17$) and was 0.25 (LSE=0.192, USE=0.325, $n=15$) for those receiving the reciprocal treatment. Seed weight was not affected by pollination treatment.

In 1990, pollination treatment affected the probability of seed set in larkspur (Table 2.12). Seed set probability was 0.45 (LSE=0.392, USE=0.512, $n=26$) for open-pollinated flowers, 0.23 (LSE=0.169, USE=0.295, $n=12$) for those receiving the pollen mixture and 0.03 (LSE=0.014, USE=0.079, $n=2$) for those pollinated heterospecifically. Seed set probabilities for open-pollinated flowers and flowers given the pollen mixture did not significantly differ from each other.

Table 2.12. Analysis of the effects of interspecific pollination on seed set and seed mass in larkspur.

	seed set, 1989	seed mass, 1989 ²	seed set, 1990
pollination treatment ¹	$F_{1,30} = 2.13$	$F_{1,29} = 0.02$	$F_{2,37} = 7.68^{**}$

^{**} $P < 0.01$

¹Inflorescence size and relative flower position were not significant as covariates and were excluded from all analyses.

²The number of seeds and ovules per flower were not significant covariates of seed mass and were excluded.

2.3.5.4.2 Monkshood

In 1989, the series 2 pollinations of monkshood led to no overall effect of pollination treatment (Table 2.13). The mean probabilities of seed set for the conspecific/heterospecific and heterospecific/conspecific treatments were 0.40 (LSE=0.363, USE=0.439, $n=40$) and 0.32 (LSE=0.283, USE=0.366, $n=32$), respectively. Seed mass was not significantly affected by pollination treatment, but monkshood seeds were much heavier in the larger meadow: seeds produced by plants in meadow A had an average dry weight of 0.15 mg (LSE=0.141, USE=0.155, $n=38$) and those from meadow B only 0.12 mg (LSE=0.109, USE=0.123, $n=32$).

In 1990, there was no detectable difference between the three pollination treatments of monkshood (Table 2.13). The probability of seed set was 0.42 (LSE=0.342, USE=0.497, $n=19$) for open-pollinated ovules, 0.14 (LSE=0.090, USE=0.200, $n=9$) for the mixed pollination and 0.07 (LSE=0.025, USE=0.158, $n=2$) for the heterospecific pollination. The extreme variation, and low sample size due to failure to set fruit, associated with these means prevented differentiation between them.

2.4 Discussion

The fundamental observation to be explained is that only about half of all ovules produced in naturally pollinated larkspur and monkshood matured into seeds. Monkshood did set significantly more seeds in the absence of larkspur (meadow B), but even then the probability of seed set was only 60%. The results suggest that both the quantity and quality of pollen received are generally adequate and that other explanations for reproductive limitation are necessary.

Table 2.13. Analysis of the effects of interspecific pollination on seed set and seed mass in monkshood.

Effects ¹	seed set, 1989	seed mass, 1989 ²	seed set, 1990 ³
pollination treatment	$F_{1,68} = 1.83$	$F_{1,66} = 0.07$	$F_{2,27} = 5.22^*$
meadow location	$F_{1,68} = 0.39$	$F_{1,66} = 11.01^{**}$	-
treatment x meadow	$F_{1,69} = 0.03$	$F_{1,66} = 0.48$	-

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

¹Inflorescence size and relative flower position were not significant as covariates and were excluded from all analyses

²The number of seeds and ovules per flower were not significant covariates and were excluded

³1990 pollinations were performed in meadow A only

2.4.1 Natural pollination and manual outcrossing

If natural levels of pollination limited seed production, then manual outcrossing should have resulted in increased seed set because manual outcrossing ensured that a sufficient quantity of pollen was deposited on the stigmas. That open-pollinated larkspur set more seeds than manually outcrossed flowers suggests some effect of the pollination treatments other than simply the quantity of pollen. Manually outcrossed flowers should have suffered no inbreeding effects relative to naturally pollinated flowers.

There are several reasons that manually outcrossed larkspur flowers set fewer seeds than those that were naturally pollinated. First, manipulation during hand pollination may have had detrimental effects because of poor estimation of stigma receptivity, use of pollen that was old or had been exposed in its anthers for too long, or the physical application of the pollen may have damaged female reproductive structures. Second, pollen applied to flowers may not have adequately sampled the pollen pool of the population.

During series 2, open-pollinated monkshood set more seed than did manually outcrossed flowers but the treatments were equally successful during series 3. The reduced success of the outcrosses may have resulted from the same factors as in larkspur, but the equal success in series 3 is confusing. However, both pollination treatments were more successful in series 3 which suggests that a factor other than pollination is more important in determining reproductive success. If environmental conditions such as available resource levels changed during the season this might cause the changes in seed set patterns.

2.4.2 Reproductive limitations and pollen quality

2.4.2.1 Effects of self-fertilization

Larkspur appears to suffer some inbreeding depression when self-pollinated. Although self-pollination and outcrossing resulted in similar seed set, seeds produced following self-pollination were smaller. Seed mass is only one aspect of the reproductive cycle that may manifest inbreeding depression, but it may be significant enough to affect viability. Monkshood is also self-compatible, with the two pollination treatments resulting in similar levels of seed set. Lack of data on seed mass for monkshood precluded examination of inbreeding depression effects on this component of reproductive success.

Both larkspur and monkshood are strongly protandrous which reduces the opportunity for self-pollination within individual flowers. Because the flowering sequence is acropetal and the pollinators tend to move up inflorescences from their point of arrival, geitonogamous pollinations are also avoided. The very high outcrossing rates indicate the effectiveness of these outcrossing mechanisms and imply that development of only half of a flower's ovules into seeds does not result from detrimental effects of self-fertilization. Should self-pollination occur, some measure of reproductive success is still assured because of self-compatibility.

2.4.2.2 Outcrossing and paternal diversity

There are strong indications that the quality and quantity of the pollen received influences seed set in some species; pollination intensity and the identity of pollen donors have been found to affect fruit set, seed set and seed mass (Marshall and Ellstrand 1986, Bertin 1990, Marshall 1991). A diverse sample of pollen gives the maternal plant the opportunity to select among the potential sires represented (Marshall and Ellstrand 1986, 1988, Snow and Spira 1991). However, this was not borne out conclusively by the multiple donor pollinations of larkspur and monkshood.

There was no change in seed set or seed mass with increasing number of pollen donors.

Other studies have also failed to show beneficial effects of multiple pollen donors. Snow (1990) performed pollinations of *Raphanus raphanistrum* using variable numbers of donors but did not find that pollen load diversity increased offspring vigor. Similarly, Sork and Schemske (1992) concluded that maternal fitness in *Chaemicrista fasciculata* was not enhanced by mixed pollen loads representing multiple outcross sources. The negative result may be due to insufficient diversity in the multiple donor pollen loads (Snow 1990). Despite the proposed significance of gametophytic competition for angiosperms (Mulcahy 1979), the effects of multiple pollen donation may be difficult to separate from other influences on fertilization success.

2.4.2.3 Competitive interactions and interspecific pollen transfer

Interspecific interactions do not significantly affect female success in larkspur and monkshood. Open-pollinated larkspur set seed with equal success in both series 1 and 2. Because series 2 occurred during a higher flowering density of monkshood than series 1 (Figure 2.2) any changes in pollinator preference, assuming no significant changes in pollinator density, should have been reflected in reduced seed set in larkspur. Monkshood flowers had a lower probability of seed set in meadow A than in meadow B in both series 2 and 3; however seed set probabilities were not influenced by changing larkspur densities but by other factors, such as nutrient resource availabilities, specific to the individual meadows. Contrary to Waser's (1978b) findings for *Ipomopsis aggregata* and *Delphinium nelsoni*, phenological overlap did not lead to reduced reproductive success in either larkspur or monkshood.

Assessment of competition for pollination is facilitated by removal of flowers from the area of competition (Campbell 1985). The distribution of plants in the

present study satisfies this criterion in terms of examining female success and the results suggest that such competition is not a major factor in the reproduction of these plants. Early flowering by larkspur does not appear to be a mechanism to avoid competition for pollination with monkshood. It is also unlikely that monkshood flowers slightly later to avoid competition with larkspur. However, the true significance of interspecific interactions and competition must also include examination of their effects on male reproductive success (Campbell 1985) but the low incidence of interspecific pollen transfer indicates that male function is unaffected as well.

The use of the two meadows provided a situation where the potentially competing plants were in close proximity, as well as a control where monkshood was free from possible reproductive interactions with larkspur. If changing plant densities of the two species affected pollinator behavior, this should have been reflected not only in seed set by the plants, but also in the occurrence of interspecific pollen transfer. Interspecific pollen transfer has been implicated repeatedly in lost reproductive success, although maternal success suffers less than paternal success (Campbell and Motten 1985, Waser and Fugate 1986, Galen and Gregory 1989, Feinsinger and Tiebout 1991).

The results of the interspecific pollinations in 1989 suggest that monkshood and larkspur plants receiving conspecific pollen, even if it was contaminated with foreign pollen, reproduced as successfully as those pollinated conspecifically. There were no significant differences between outcrosses and either of the two pollen mixtures. In monkshood, outcrossed flowers set more seeds than did flowers that received both heterospecific and conspecific pollen but the latter flowers still set seed with a probability of 0.32.

Similar pollinations in 1990 showed slightly different results. Mixed pollinations in monkshood set fewer seed than outcrosses as might be expected if foreign pollen clogged stigmas, interfered with pollen tube growth or attempted to fertilize ovules in a manner that spoils them. Larkspur showed the expected pattern more clearly. Heterospecific pollinations were the least successful, with the mixed pollination treatment having the intermediate result. This suggests that the foreign pollen creates some interference but that the conspecific pollen is able to fertilize the ovules. The fact that some heterospecific pollinations resulted in seeds being produced in either species is more likely due to accidental contamination than to truly successful seed set. Contamination may have been caused by wind-borne pollen or prior contamination of the the anthers used in hand pollinations by pollinators.

The incidence of interspecific pollen transfer between larkspur and monkshood was very low. The stigmas of both open-pollinated monkshood and larkspur had insignificant amounts of interspecific pollen on them. Therefore, limited seed set in monkshood and larkspur must be attributable to other causes.

The results of interspecific pollen transfer may be species specific or depend on the timing of heterospecific pollination. On the one hand, seed set in *Diervilla lonicera* was strongly depressed by mixed pollination with heterospecific pollen (Thomson *et al.* 1981). Galen and Gregory (1989) found that female function in *Polemonium viscosum* was inhibited by application of foreign pollen prior to conspecific pollination. Similarly, conspecific pollination was not even possible in *Ipomopsis aggregata* following earlier interspecific pollination (Waser and Fugate 1986). On the other hand, if conspecific and heterospecific pollen were applied simultaneously in a mixture, then seed set in *Ipomopsis aggregata* was unaffected (Waser and Fugate 1986). Kwak and Jennersten (1986) showed that seed set in *Viscaria vulgaris* was not adversely affected by receipt of mixed pollen loads.

2.4.3 Other influences on reproductive success

Plants have limited resources with which to grow, produce reproductive structures and nourish developing seeds and fruits. Several aspects of plant size affect seed set and seed mass in larkspur and monkshood and this may be attributed to limited levels of nutritional resources available for reproduction. Specifically, effects of meadow location, inflorescence size, relative flower position and ovule number per flower indicate that seed set and seed mass may be determined as much by resource levels as by pollination effects.

Several analyses indicate that pollination is not the sole determinant of seed set and seed mass but that it may interact with resource availability to determine reproductive success. Zimmerman and Pyke (1988), in outlining appropriate experimental protocol for examining resource and pollen limitation in plants, stated that if a small fraction of flowers on a plant were liberally pollinated relative to other flowers, then these flowers may act as disproportionately strong resource sinks. This means that there is a balance between pollen and resource limitation of seed set that may be tilted by manipulation.

The analysis of outcrossed and open-pollinated larkspur (Table 2.7a) produced an interaction between relative flower position, pollination treatment and pollination series. Open-pollinated larkspur, although setting a larger proportion of their seeds, may have had a more uniform distribution of pollen within the inflorescence than manually pollinated plants. Given uniform open pollination, flowers lower in the inflorescence may have been allocated a greater proportion of resources because they set fruit first thus generating a negative effect of flower position (Table 2.7b).

Seed mass in both open-pollinated larkspur (Table 2.4) and monkshood (Table 2.5) was related to various measures of plant size. Seed mass increased with inflorescence size in larkspur which suggests that larger plants, which have more

flowers, also produce larger seeds as well as more seeds. Seed weight was positively correlated with ovule number in open pollinated monkshood. This may mean that plants with the resources to produce many ovules can also adequately nourish these ovules, to a greater extent than plants that produce only a few ovules.

Relative flower position influenced seed mass differently in larkspur and monkshood. Seed mass increased with relative flower position in larkspur (2.3.4.1, Table 2.4), but decreased with relative flower position in monkshood in meadow B (2.3.4.2, Table 2.5). If a limited amount of nutritional resources must be allocated to developing seeds, then the position of a flower within an inflorescence may influence its strength as a resource sink (Van Steveninck 1957). For example, flowers near the bottom of the inflorescence flower first and set seeds before other flowers become demanding resource sinks. Differences in the number of developing seeds within a fruit, resulting from different levels of pollination, may also determine patterns of resource allocation (Zimmerman and Pyke 1988).

Differences in the reproductive success of monkshood seem to be due to characteristics of individual plants. Monkshood inflorescences were larger in meadow A than in meadow B (Table 2.2) which initially suggests that plants experienced different growing conditions and nutrient levels. The larger plants of meadow A set a lower proportion of seeds per flower than those in meadow B (Table 2.5, 2.8), but this was compensated for by the larger inflorescence size. In fact, total seed production per plant (ignoring pollination series and whether flowers were open-pollinated or manually outcrossed) was identical in meadows A and B. Total seed production per plant was (mean \pm SE) 139.0 \pm 9.04 in meadow A and 140.9 \pm 7.54 in meadow B. Perhaps nutrient availability placed the ultimate limit on seed set, and in 1989 monkshood in meadows A and B had similar levels of resources available on a

per plant basis. The fact that monkshood inflorescences were larger earlier in the season indicates that nutrient conditions may change.

Seeds from outcrossed monkshood in meadow B were lighter than those from meadow A and in meadow B seed mass decreased with relative flower position (Table 2.8). Both of these results indicate that resources available to the developing seeds became limiting, at least in meadow B. This suggests that there was an a priori difference between the two fields with monkshood in terms of plant quality. The quality of the plants may be measured in terms of the amount of resources that the plants can direct to their developing seeds.

There is a problem with trying to relate seed set and reproductive success to other measures of plant size and vigour. If individual flowers are pollen limited in their seed set then the plant may compensate by producing more flowers and then using selective abortion to regulate the ultimate total seed set by maturing the fruits that happen to have the most fertilized ovules (Bawa and Beach 1981, Stephenson 1981). This means that pollen receipt and resource availability may each limit reproduction or they may do so simultaneously.

The results indicate that both pollen receipt and measures of plant resource levels can explain reproductive success in larkspur and monkshood. The true limitations of reproduction in monkshood and larkspur will be understood by simultaneously examining whether pollen receipt or other resources limit reproductive effort (see Chapter 3).

3.1 Assessment of limitation of female function by nutrient resources and pollen receipt

All plants are in some manner limited in their reproductive output, so that plants often produce more flowers than fruits and more ovules than seeds (Stephenson 1979, Willson 1979, Lloyd *et al.* 1980, Udovic 1981, Aker 1982, Bawa and Webb 1984, Sutherland and Delph 1984, Sutherland 1986, Wiens *et al.* 1989, Allen and Wilson 1992). Limitations on plant reproduction may result from several causes. First, if the quality or quantity of pollen received limits the production of seeds or fruit or some other measure of reproductive success then reproduction is pollen limited. Inadequate pollination commonly limits plant reproduction (Bierzychudek 1981, Arnold 1982, Snow 1982, Morse and Fritz 1983, Travis 1984, Campbell 1985, 1987, 1991, Campbell and Motten 1985, Garwood and Horvitz 1985, Hainesworth *et al.* 1985, Paige and Whitham 1987, Bronstein 1988, Horvitz and Schemske 1988a, Whelan and Goldingay 1989, Berry and Calvo 1991, Johnston 1991b, Kwak and Jennersten 1991, Fox 1992, Karoly 1992). In general it is demonstrated by manual pollination that increased the quantity of pollen received, increased both fruit and seed set in *Staphylea trifolia* (Garwood and Horvitz 1985), *Calathea ovandensis* (Horvitz and Schemske 1988a), *Ipomopsis aggregata* (Hainesworth *et al.* 1985), *Lobelia cardinalis* and *L. siphilitica* (Johnston 1991b) and *Lupinus nanus* (Karoly 1992). Second, the ability of a plant to complete its maternal function following fertilization of ovules may be subject to resource limitation. Resource limitation has been demonstrated by decreasing the number of sinks drawing on a plant's resources by removing developing fruits (Stephenson 1980, Willson and Burley 1983, McCall and Primack 1985, Queller 1985, Galen and Weger 1986, Gorchov 1988, Ehrlén 1992), by decreasing the total level of resources in a plant by removing leaves (Lee and Bazzaz 1980, 1982, Stephenson 1980, Willson and Price 1980), by manipulating the

number of developing seeds (LaLonde and Roitberg 1989) or developing fruits (Stephenson 1984) via different levels of pollination or by enhancing nutrient levels using mineral fertilizers (Willson and Price 1980, Queller 1985) or water (Delph 1986). Shortages in either component of the reproductive process may limit the number or quality of seeds by preventing fertilization and subsequent maturation of ovules.

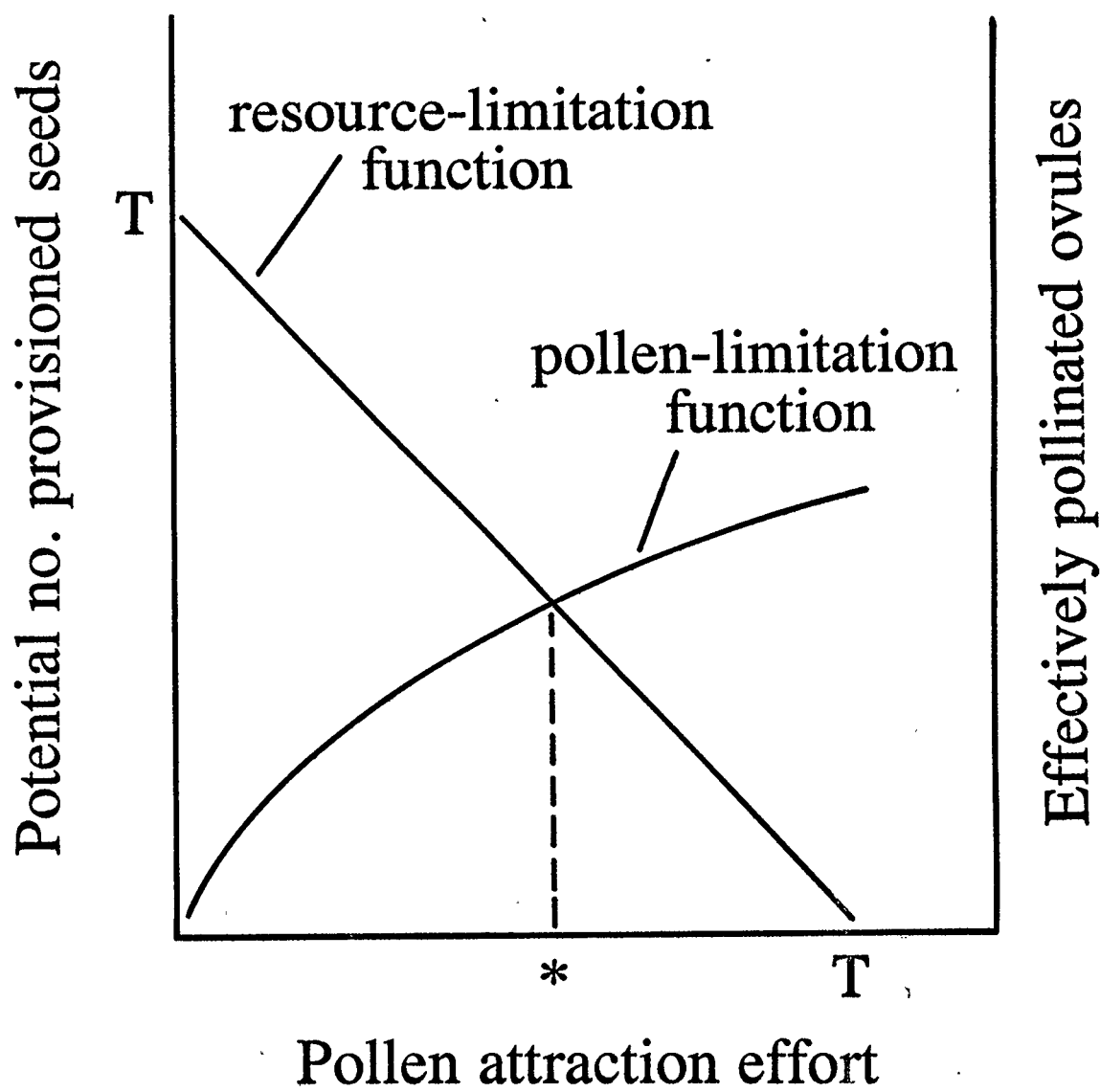
3.1.1 Pollen and resource limitation in plants

There is not a clear dichotomy between resource and pollen limitation. Interactions between reproductive influences may be complex and these processes should be considered over a long period, comparable to the life of the plant, because short- and long-term limitations may not be identical. The following examples demonstrate that resources and pollination may interact to limit reproductive success. Janzen *et al.* (1980) suggested that increased fruit set in *Encyclia cordigera* following hand-pollination may not demonstrate pollen limitation if the plants were never exposed to high levels of pollination previously. The resulting high fruit set may diminish the plants' resources so that survival and/or reproductive effort in subsequent seasons are reduced. Petersen *et al.* (1982) indicated that resource and pollen limitation are not easily separated in the perennial *Chilopsis linearis* because current pollination levels may affect the allocation of resources to current reproductive effort or to plant growth and future reproduction. This trade-off was clearly demonstrated by Paige and Whitham (1987) in *Ipomopsis aggregata* which switched from semelparity to iteroparity when pollen was limiting. *Thalictrum thalictroides* experienced changing environmental conditions that shifted the relative levels of pollen and resource limitation during the flowering period (Lubbers and Christensen 1986). In *Encyclia krugii*, increased fruit set following hand-pollination caused the cessation of further flower production (Ackerman 1989). Manual pollination

increased fruit set of *Aspasia principissa* six-fold but subsequent fruits were smaller as were the inflorescences of these fruit-producing individuals in the subsequent season (Zimmerman and Aide 1989). Similarly, hand-pollinations increased fruit set in *Epidendrum ciliare*, but total seed crop per plant decreased with increasing fruit set (Ackerman and Montalvo 1990). These plants that experienced heavy fruit set had fewer, smaller inflorescences during the next season and showed less vegetative growth as well (Ackerman and Montalvo 1990). Resource and pollen limitation of *Lathyrus vernus* differed in their relative importance between years (Ehrlén 1992). Clearly, pollen and resource limitation are not mutually exclusive.

Haig and Westoby (1988a) concisely summarized the operation of trade-offs constraining a plant's reproductive effort. Based on the economic analogy of Bloom *et al.* (1985), they outlined how a given reproductive effort may be allocated to plant features (e.g. inflorescence size, floral rewards) that affect pollen import, or to the provisioning of developing seeds. For any particular division of resources among pollen attraction and ovule nourishment, either pollen receipt or available resources will limit reproductive success. The number of seeds that can be matured will decrease when a greater proportion of total reproductive effort is allocated to attracting pollen. Conversely, the number of ovules that are pollinated will increase. The lesser of these two functions should limit the reproductive success of the plant during any reproductive episode (Figure 3.1). An individual acting optimally would allocate resources such that reproduction is equally limited by both functions (Bloom *et al.* 1985, Haig and Westoby 1988a), thereby ensuring a fitness return for all resources invested in reproduction. For example, with insufficient transfer of pollen some of the resources allocated to producing female structures will have been wasted. Hence, in the long term, a species should be equally limited by both pollen receipt and resources (Haig and Westoby 1988a).

Figure 3.1. Resource and pollen limitation of plant reproductive effort. Total maternal resources, T , are divided between pollen attraction and provisioning of developing seeds. As pollen attraction effort increases, so does the number of effectively pollinated ovules but the number of provisioned seeds decreases. For any allocation of reproductive resources, seed set will be determined by the lesser of the two functions. At * both functions equally limit reproduction. (From Haig and Westoby 1988a.)



3.1.2 Size and number trade-offs in seed production

Seed size is an important factor in plant reproduction because it may significantly affect offspring survival (Wulff 1986). If there are inadequate resources to mature all seeds and fruits fully then there are several alternatives. The plant may fully mature a limited number of fruits or seeds, implying that some mature at the expense of others. Alternatively, available resources can be partitioned among all developing structures which may result in all fruits and seeds being smaller than those that are fully matured.

Smith and Fretwell (1974) provided one of the first theoretical examinations of size versus number trade-offs in offspring. Their model is most appropriate for organisms producing numerous, relatively small offspring that receive no parental care. This certainly is the case in most plant species which typically provide no parental care beyond the initial provisioning of seeds and which often produce many propagules, each representing only a small investment. Smith and Fretwell (1974) demonstrated that although offspring fitness may increase with parentally allocated resources (i.e. larger seeds), parental fitness will be maximized for some optimum allocation of energy and resources to each individual offspring. It is beneficial for a plant to produce more offspring as long as their average fitness does not decline (Lloyd 1979).

The Smith and Fretwell (1974) model predicts that seed size within a brood should vary little if all offspring are identical. Increased resource availability should lead to more, rather than larger, seeds (Haig and Westoby 1988b). However, seed size is not constant within species or within individual plants (Michaels *et al.* 1988, Fenster 1991) and may be determined by genetic quality, present resource availability or past resource fluctuations and reproductive history. For example, Temme (1986) predicted that genetic differences among offspring, that are detectable by the maternal

parent, provide the basis for adaptive differential provisioning of developing seeds. Defoliation of *Abutilon theophrasti* reduced seed production (Lee and Bazzaz 1980) but similar treatment of *Rumex crispus* affected seed size (Maun and Cavers 1971).

3.1.3 Abortion of fruits and ovules

Plants may respond to deficient resource levels by failing to mature all of the fruits and seeds initiated. Abortion of fruits and ovules within fruits is well-documented (Stephenson 1981 and references therein). Fruit abortion also provides a means by which plants may control the quality of offspring produced (Stephenson and Winsor 1986) and it may be executed on the basis of the identity of the fertilizing pollen (Bertin 1982a, Bookman 1984, Temme 1986, Haig 1990). Resource limitation would increase the selective pressure to discriminate among pollen parents. Conversely, fruits with only a small proportion of ovules setting seed, because of inadequate pollen receipt, should be aborted when resources are limiting because they represent a poor return on resources invested (McDade and Davidar 1984). Fruits that contain relatively few developing seeds may experience disproportionately high abortion (Bertin 1982b).

Abortion is ultimately a response to available resources and manipulation of resource levels within plants affects abortion levels. Defoliation of *Epilobium montanum* increased abortion of ovules (Harper and Wallace 1987). Stephenson (1980) defoliated branches of *Catalpa speciosa* causing increased fruit abortion within those branches which subsequently increased the probability of maturation for the remaining fruits on other branches.

3.1.4 Objectives

The previous chapter examined in detail whether pollination affected reproductive success in larkspur and monkshood. Pollen quality and quantity were not limiting for reproduction but it was possible that resources were.

This study will determine whether larkspur and monkshood are pollen or resource limited within a single reproductive season. The effects of resource enhancement, supplemental pollination and of these treatments in combination, on fruit set, seed set and seed abortion will be compared to reproductive success experienced in unmanipulated plants.

3.2 Methods

3.2.1 Experimental design

To examine potential resource and pollen limitation in monkshood, *Aconitum delphinifolium*, and larkspur, *Delphinium glaucum*, I conducted an experiment that involved fertilization and supplemental pollination of 600 plants: 300 of each species. Two types of fertilizer were used in the experiment. Plant Prod® Flowering Plant Fertilizer (15-30-15) encourages blooming and Plant Prod® Tomato and Vegetable Fertilizer (15-15-30) promotes fruit development. I will henceforth refer to these phosphorus- and potassium-rich fertilizers as P-rich and K-rich, respectively.

The plants were randomly assigned to the treatment groups illustrated in Figure 3.2. Groups of 20 plants were assigned to treatments that differed in the type and amount of fertilizer applied and in the application of supplemental pollen. Each of the two fertilizers was applied at the recommended dosage of 3.5 ml/l and at a less concentrated dosage of 2.5 ml/l. Fertilized plants received a 1-l application of aqueous fertilizer solution once every two weeks. Mixtures of the two fertilizers were also applied at high and low concentrations. All plants experienced natural pollination and half of the plants received pollen supplemental to that acquired naturally. The six

Figure 3.2. Experimental design for examination of resource and pollen limitation in larkspur and monkshood. Numbers within cells indicate the number of plants assigned to particular fertilizer treatments. The indicated distribution of plants among fertilizer treatments was repeated for both supplemental and open-pollinations (300 plants per species).

		P-rich concentration		
		0	Low	High
K-rich concentration	0	n=30	n=20	n=20
	Low	n=20	n=20	
	High	n=20		n=20

fertilizer treatments, combined with manual or natural pollination, produced 12 treatment groups of 20 plants each for each species. Additionally, another 60 unfertilized plants were examined for each species as a control. Half of these were naturally pollinated whereas the others received supplemental pollen applied manually.

3.2.2 Fertilizations and pollinations

A single application of fertilizer to a plant consisted of 1-ℓ of solution mixed at the appropriate dose. The plants emerge from a layer of duff, moss and grass up to 10 cm thick. I penetrated this layer with a finger and poured the fertilizer solution in slowly to allow most of it to reach the soil surrounding the plants' roots rather than running off. One ℓ was sufficient to wet most of the root system, ensuring ample opportunity for applied fertilizers to be absorbed. All plants receiving fertilizer were treated every two weeks commencing when the flowers were still buds. Eventually, all fertilized plants received three applications which were administered on 23 July, 6 August and 20 August 1990.

I attempted to pollinate all flowers in the inflorescences of manually pollinated plants. Zimmerman and Pyke (1988) indicated that ensuring ample pollen receipt for only a small fraction of the flowers in an inflorescence might simply turn these flowers into stronger resource sinks, thereby failing to resolve the relative impact of pollen versus resource limitation. Given the respective inflorescence sizes (see 2.2.1), this was much easier to achieve for monkshood than for larkspur. Almost total pollination of inflorescences was achieved in monkshood. In larkspur, an average of 40% of the flowers in an inflorescence were supplementally pollinated.

A single pollination involved manually pollinating a flower in female phase with pollen from at least two donor males. During manual pollination I used a single dehiscent anther from a male flower as a brush to pollinate the receptive stigmas.

Stigmas are receptive when their tips splayed slightly revealing an adhesive surface. I surveyed all the plants requiring manual pollination every three to four days and pollinated all female flowers encountered. Given the duration of female phase in these species (see 2.3.1) I had ample opportunity to pollinate most receptive flowers. These pollinations supplemented natural pollen receipt and involved no emasculations or pollinator exclusions. I was able to identify each flower in an inflorescence by counting up from the base of the raceme. This allowed me to keep track of which flowers were supplementally pollinated.

3.2.3 Harvest and examination of fruit

Four fruits were collected from each plant on 29 August 1990 and treated as per 2.2.7. Fruits were dissected under a stereo dissecting scope (60X magnification) and the fate of each ovule determined. Each ovule remained undeveloped or developed into a seed that was matured or subsequently aborted. A well-developed seed was round and firm within a loose, wrinkled seed coat. Aborted seeds were only partially filled or were black and mushy. All seeds collected were dried overnight at 60°C and weighed to determine dry mass.

3.2.4 Statistical analysis

As in the previous chapter (see 2.2.8), I used analyses of covariance to assess sources of variation in fruit set, seed set per fruit, total seed set per plant, seed abortion and seed mass. The experimental design includes several empty cells corresponding to mixtures of fertilizers at different concentrations (Figure 3.2). This necessitated the use of Type IV sums of squares in the analyses (Milliken and Johnson 1984, SAS 1987). This had the drawback of not allowing me to estimate marginal means. To facilitate presentation, figures depicting fertilizer effects are based on only the three cells representing the effect of one fertilizer in the absence of the other. The

trends shown in these figures are representative of the overall effects found in the analyses.

To eliminate within-plant effects from the analyses, I calculated the probability of seed set within each flower and then determined an average value for all the harvested flowers within a plant. I estimated total seed set per plant as the product of the average probability of seed set and inflorescence size.

Several covariates relating to plant size were included in the analyses. Inflorescence size and number of leaves per plant were used in most analyses. These covariates were measured at the time of harvest. Analyses of seed mass and abortion included the number of seeds and ovules per flower. When covariates affected treatment groups homogeneously I report treatment means as though all plants were measured at the mean value of the covariate. In contrast, when a covariate affected treatment groups heterogeneously, I report the partial regression coefficients for the covariate within each treatment.

3.3 Results

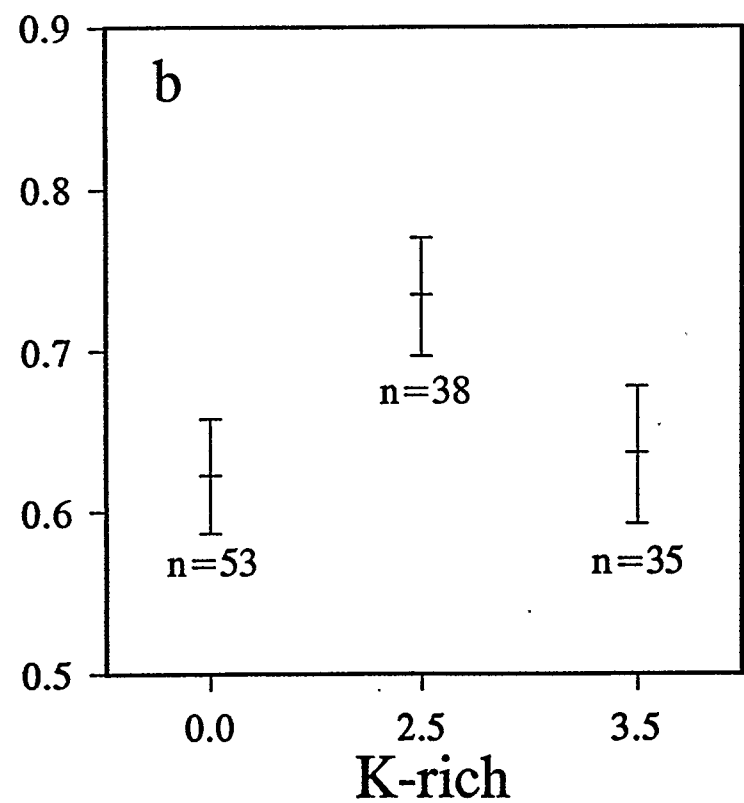
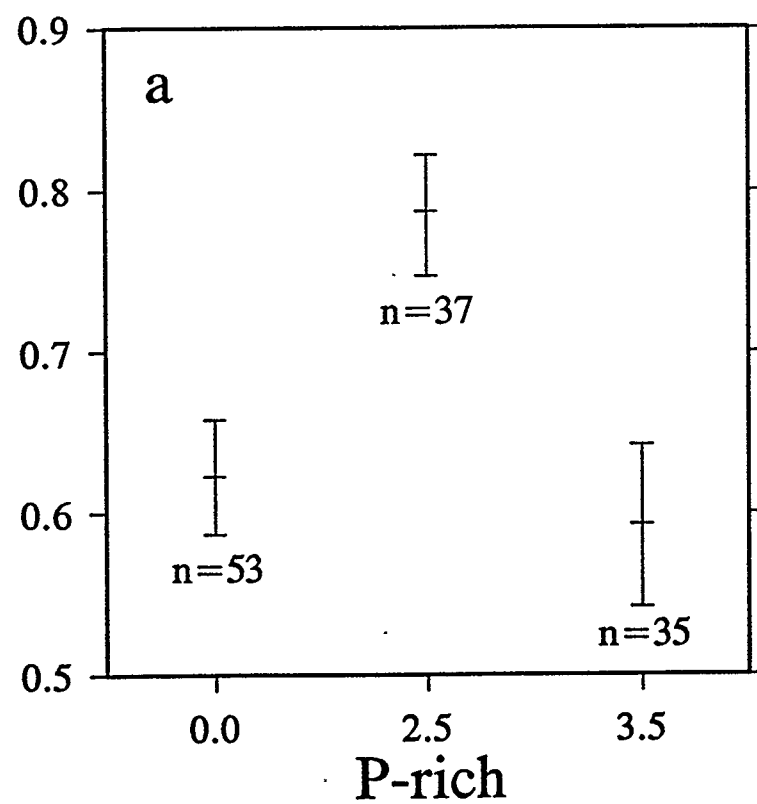
3.3.1 Larkspur

3.3.1.1 Fruit set

Larkspur appears to be resource limited because not all flowers developed fruits (mean fruit set probability, 0.62, LSE=0.587, USE=0.658), not all ovules developed into seeds (mean seed set probability, 0.46, LSE=0.427, USE=0.495), and fertilizer treatments increased both fruit and seed set. Fruit set for unfertilized and highly fertilized plants was similar, but plants receiving a low concentration application of P-rich fertilizer set a higher proportion of fruit (Figure 3.3a; Table 3.1). The trend was similar for K-rich fertilizer, but the overall effect was not significant (Figure 3.3b; Table 3.1). Supplemental pollination did not enhance fruit set (Table 3.1).

Figure 3.3. Effects of a) P-rich and b) K-rich fertilizer on the mean (\pm SE) probability of fruit set in larkspur.

Proportion of flowers developing fruit



Fertilizer concentration (ml/l)

Table 3.1 Analysis of the effects of fertilization and supplemental pollination on fruit set, seed set per flower and total seed set per inflorescence in larkspur.

Effects	fruit set ¹	seed set ²	total seed set
P-rich fertilizer	$F_{2,255} = 5.24^{**}$	$F_{2,232} = 3.72^*$	$F_{2,230} = 6.35^{**}$
K-rich fertilizer	$F_{2,255} = 2.77$	$F_{2,232} = 0.96$	$F_{2,230} = 0.87$
P-rich x K-rich	$F_{2,255} = 2.20$	$F_{2,232} = 2.69$	$F_{2,230} = 2.88$
pollination treatment	$F_{1,255} = 2.01$	$F_{1,232} = 2.04$	$F_{1,230} = 1.56$
P-rich x pollination	$F_{2,255} = 0.48$	$F_{2,232} = 2.41$	$F_{2,230} = 2.19$
K-rich x pollination	$F_{2,255} = 1.11$	$F_{2,232} = 2.63$	$F_{2,230} = 3.06^*$
P x K x pollination	$F_{2,255} = 1.48$	$F_{2,232} = 2.21$	$F_{2,230} = 1.91$
inflorescence size	-	-	$F_{1,230} = 24.30^{***}$
number of leaves	-	$F_{1,232} = 12.06^{***}$	$F_{1,230} = 10.48^{**}$
ovules per flower	-	-	$F_{1,230} = 434.92^{***}$

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

¹Inflorescence size and number of leaves per plant were not significant covariates and were excluded.

²Inflorescence size was not a significant covariate and was excluded.

3.3.1.2 Seed set

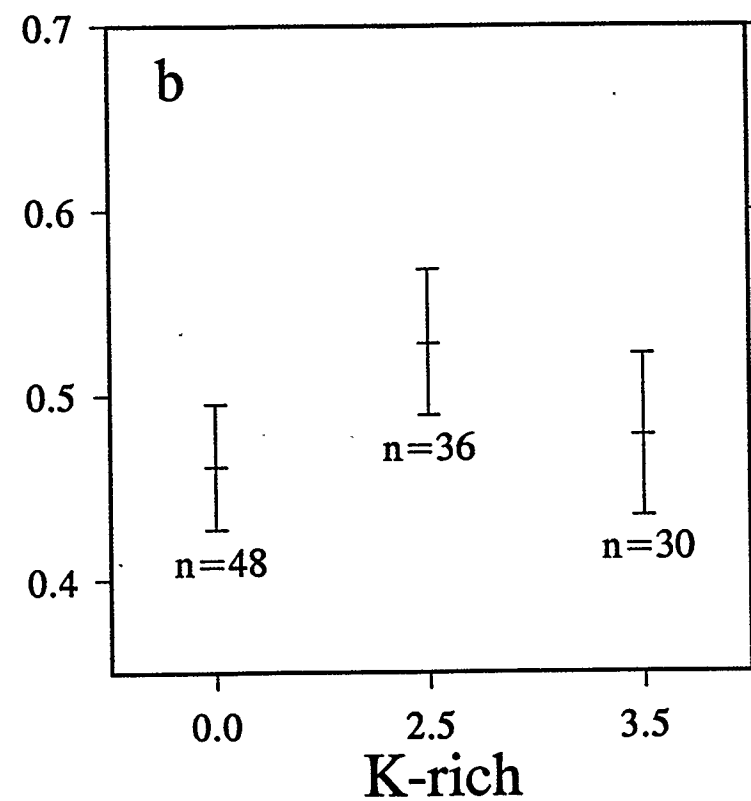
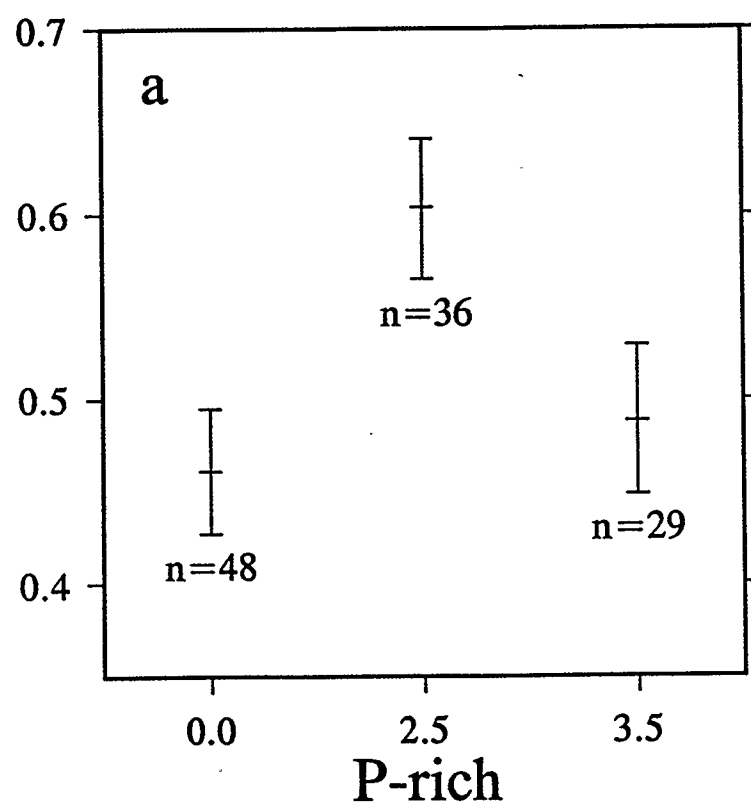
As with fruit development, seed set was highest for low-concentration fertilizer treatments (Figure 3.4a). Probability of seed set within larkspur fruits was increased by the application of P-rich fertilizer (Table 3.1). The general trend was similar for K-rich fertilizer, but was not significant (Figure 3.4b). Supplemental pollination did not affect seed set. Probability of seed set was positively related to the number of leaves on a plant ($b \pm s_b = 0.049 \pm 0.0159$, $t_{231} = 3.11$, $P < 0.01$).

P-rich fertilizer significantly affected the total number of seeds set by larkspur plants (Table 3.1). The highest total seed production resulted from low-concentration fertilizer application (Figure 3.5a): these plants produced over 30% more seeds than unfertilized and highly fertilized plants. The trend was similar, but non-significant, for the direct effect of K-rich fertilizer (Figure 3.5b). Supplemental pollination did not affect total seed set directly. However, there was an interaction between level of K fertilization and pollination treatment: in heavily fertilized plants, total seed set increased with supplemental pollination, but pollination treatment made no difference for unfertilized and lightly fertilized plants. Total seed set was negatively related to inflorescence size ($b \pm s_b = -0.26 \pm 0.054$, $t_{230} = -4.93$, $P < 0.001$) and positively related to the number of leaves on a plant ($b \pm s_b = 0.25 \pm 0.078$, $t_{230} = 3.24$, $P < 0.01$).

Main treatments had no direct effect on seed weight (Table 3.2); however there were interactions between both P-rich fertilizer and pollination treatment and K-rich fertilizer and pollination treatment (Table 3.2). At high concentrations of P-rich fertilizer, supplemental pollination decreased seed mass, whereas supplemental pollination increased seed mass at low concentrations of P-rich fertilizer and in the unfertilized group. Supplemental pollination reduced seed mass in plants treated with both high and low concentrations of K-rich fertilizer. Unfortunately, individual

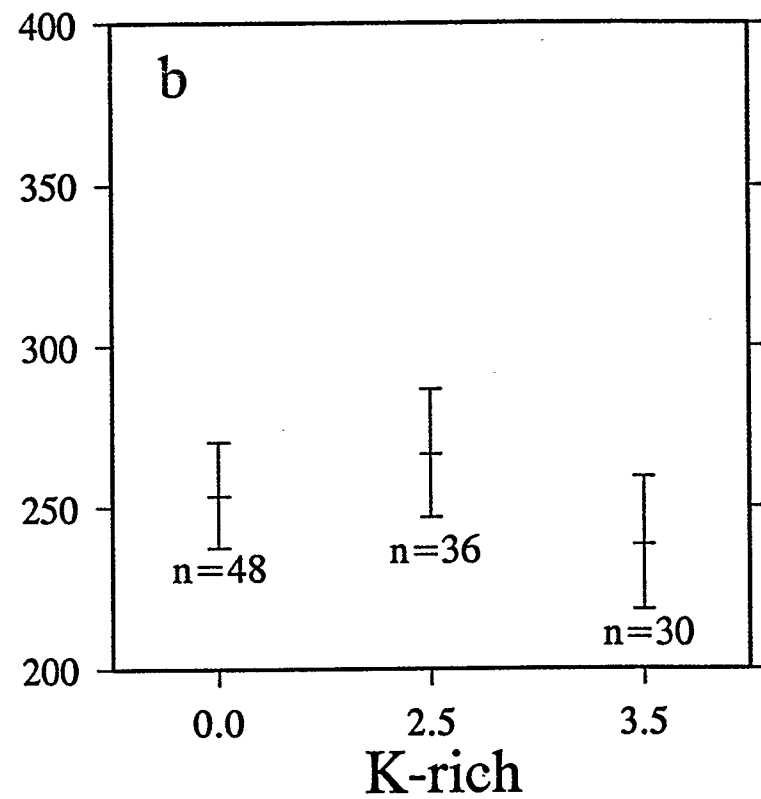
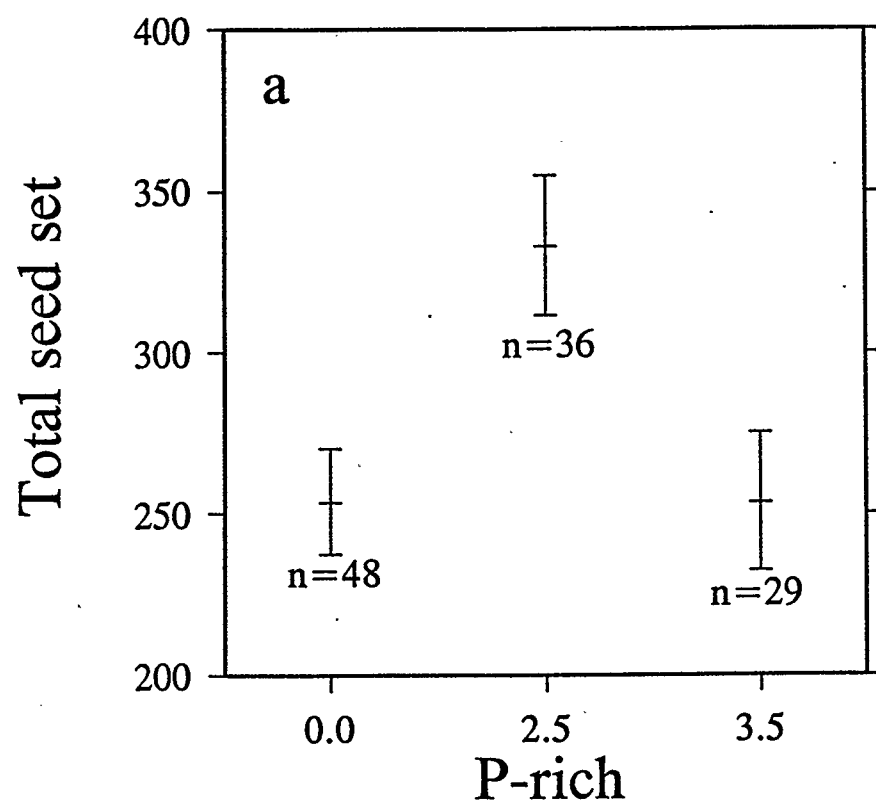
Figure 3.4. Effects of a) P-rich and b) K-rich fertilizer on the mean (\pm SE) probability of seed set per flower in larkspur.

Proportion of ovules setting seed



Fertilizer concentration (ml/l)

Figure 3.5. Effects of a) P-rich and b) K-rich fertilizer on the mean (\pm SE) total seed set per plant in larkspur.



Fertilizer concentration (ml/l)

Table 3.2 Analysis of the effects of fertilization and supplemental pollination on seed mass in larkspur.

Effects ¹	test statistic
P-rich fertilizer	$F_{2,230} = 0.65$
K-rich fertilizer	$F_{2,230} = 2.27$
P-rich x K-rich	$F_{2,230} = 1.39$
pollination treatment	$F_{1,230} = 0.42$
P-rich x pollination	$F_{2,230} = 3.90^*$
K-rich x pollination	$F_{2,230} = 3.82^*$
P x K x pollination	$F_{2,230} = 1.93$
seeds per flower	$F_{1,230} = 17.49^{***}$
ovules per flower	$F_{1,230} = 16.55^{***}$

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

¹Inflorescence size and number of leaves per plant were excluded as covariates.

components of these interactions were not estimable. Both mean number of seeds and mean number of ovules had strong positive effects on seed weight ($b \pm s_b = 0.0061 \pm 0.00145$, $t_{230} = 4.18$, $P < 0.001$ and $b \pm s_b = 0.0089 \pm 0.00218$, $t_{230} = 4.07$, $P < 0.001$ respectively).

3.3.1.3 Seed abortion

Treatment with P-rich fertilizer significantly affected the probability of seed abortion in larkspur (Table 3.3). The probability of seed abortion for plants treated with a low-concentration of P-rich fertilizer was 0.18 (LSE=0.144, USE=0.216) whereas unfertilized plants aborted seeds with a probability of 0.28 (LSE=0.236, USE=0.334), and heavily fertilized plants with a probability of 0.23 (LSE=0.183, USE=0.288).

There was a significant interaction between K-rich fertilizer and pollination treatment (Table 3.3). Within each fertilizer treatment, supplemental pollination resulted in lower probabilities of seed abortion. Naturally-pollinated, heavily fertilized plants had the highest probability of abortion (mean=0.35, LSE=0.283, USE=0.417) whereas the lowest probability was for supplementally-pollinated, heavily fertilized plants (mean=0.16, LSE=0.118, USE=0.216).

The interaction between leaf number per plant and pollination treatment (Table 3.3) was caused by the significant difference between the partial regression coefficients for the two groups ($t_{245} = -2.03$, $P < 0.05$). The partial regression coefficient for naturally-pollinated plants did not differ from zero ($b \pm s_b = -0.04 \pm 0.034$, $t_{245} = -1.26$, $P > 0.2$) whereas the relation was significantly negative for supplementally-pollinated plants ($b \pm s_b = -0.15 \pm 0.042$, $t_{245} = -3.64$, $P < 0.001$).

Table 3.3 Analysis of the effects of fertilization and supplemental pollination on seed abortion in larkspur.

Effects ¹	test statistic
P-rich fertilizer	$F_{2,245} = 5.41^{**}$
K-rich fertilizer	$F_{2,245} = 0.40$
P-rich x K-rich	$F_{2,245} = 1.62$
pollination treatment	$F_{1,245} = 2.12$
P-rich x pollination	$F_{2,245} = 0.87$
K-rich x pollination	$F_{2,245} = 4.67^*$
P x K x pollination	$F_{2,245} = 2.61$
number of leaves	$F_{1,245} = 13.13^{***}$
leaves x pollination	$F_{1,245} = 4.13^*$

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

¹Inflorescence size was excluded as a covariate.

3.3.2 Monkshood

Data on fruit set per monkshood inflorescence were not collected because I observed that almost all flowers developed into fruits. Therefore, I anticipated that fertilization treatments would potentially impact on seed set per flower rather than fruit set. Additionally, when flowers failed to mature fruits, the cause was predominantly fruit abortion resulting from predation by unidentified Dipteran larvae.

3.3.2.1 Seed set

Although on average the probability of seed set of monkshood ovules was only 0.56 (LSE=0.529, USE=0.594), seed set in monkshood was not affected by any of the experimental manipulations (Table 3.4). Neither fertilizer treatment nor supplemental pollination affected the probability of seed set. As with larkspur, a low concentration application of P-rich fertilizer tended to increase seed set but this trend was not significant (Figure 3.6a). No increase at all was evident in plants treated with K-rich fertilizer (Figure 3.6b). Probability of seed set was positively related to the number of leaves on a plant ($b \pm s_b = 0.091 \pm 0.0420$, $t_{207} = 2.18$, $P < 0.05$).

Main treatment effects also failed to affect seed weight (Table 3.4). However, seed weight was positively related to the mean number of seeds produced per flower ($b \pm s_b = 0.0050 \pm 0.00078$, $t_{205} = 6.37$, $P < 0.001$). Inflorescence size had a negative effect on seed weight ($b \pm s_b = -0.012 \pm 0.0051$, $t_{205} = -2.30$, $P < 0.05$); larger inflorescences produced smaller seeds.

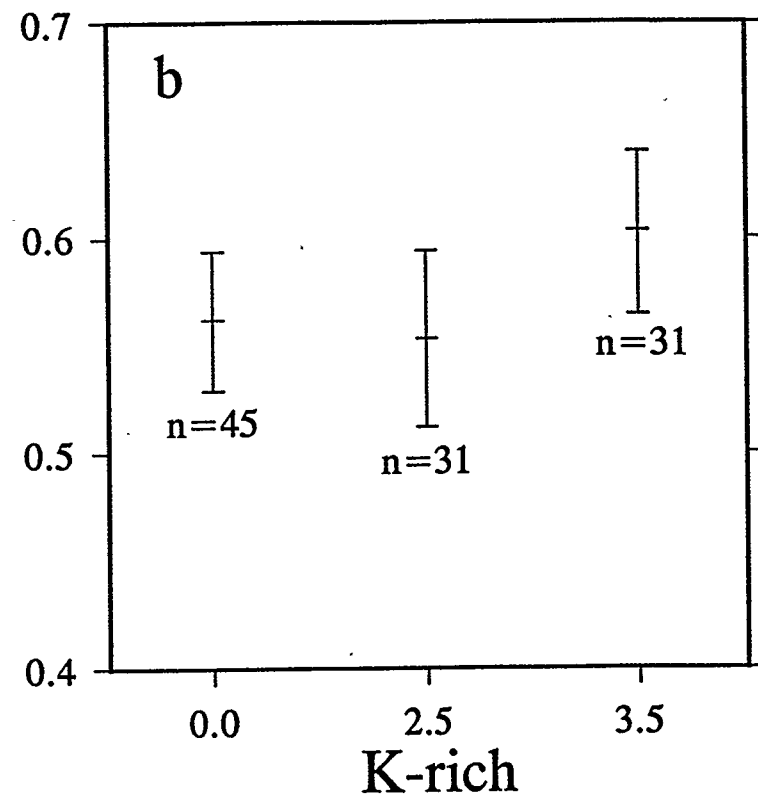
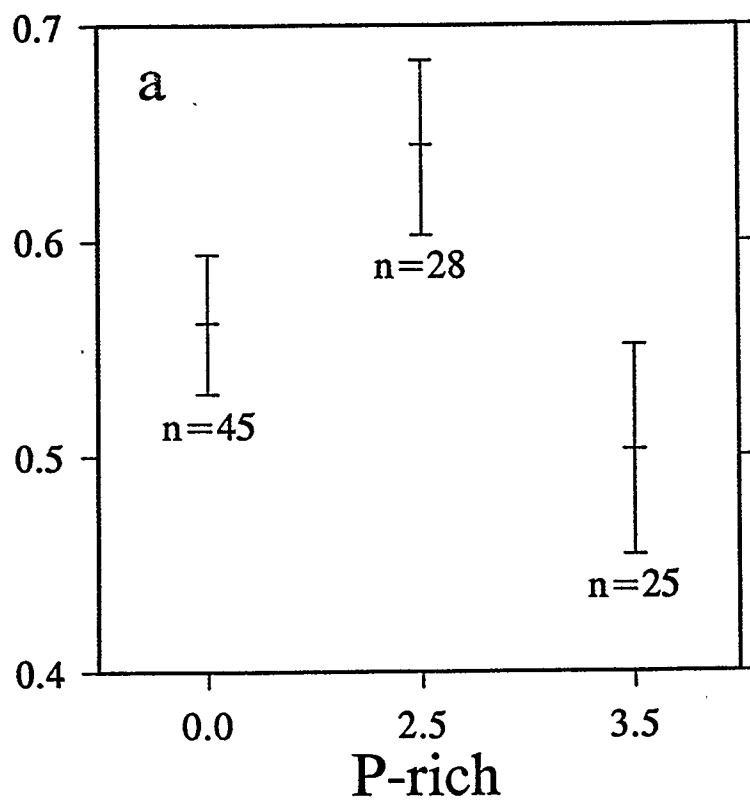
Total seed set by monkshood plants was not analyzed because the fruit set component of total seed set was not measured.

3.3.2.2 Seed abortion

Seed abortion in monkshood depended on complex interactions between main treatment effects and covariates (Figure 3.7; Table 3.5). A posteriori comparisons

Figure 3.6. Effects of a) P-rich and b) K-rich fertilizer on the mean (\pm SE) probability of seed set per flower in monkshood.

Proportion of ovules setting seed



Fertilizer concentration (ml/l)

Table 3.4 Analysis of the effects of fertilization and supplemental pollination on seed set per flower and seed mass in monkshood.

Effects	seed set ¹	seed mass ²
P-rich fertilizer	$F_{2,207} = 3.00$	$F_{2,205} = 0.29$
K-rich fertilizer	$F_{2,207} = 0.43$	$F_{2,205} = 0.63$
P-rich x K-rich	$F_{2,207} = 0.21$	$F_{2,205} = 2.70$
pollination treatment	$F_{1,207} = 0.33$	$F_{1,205} = 1.38$
P-rich x pollination	$F_{2,207} = 1.60$	$F_{2,205} = 1.13$
K-rich x pollination	$F_{2,207} = 1.47$	$F_{2,205} = 1.43$
P x K x pollination	$F_{2,207} = 0.38$	$F_{2,205} = 2.09$
number of leaves	$F_{1,207} = 4.73^*$	-
seeds per flower	-	$F_{1,205} = 40.55^{***}$
inflorescence size	-	$F_{1,205} = 5.28^*$

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

¹Inflorescence size was not significant as a covariate and was excluded.

²Number of leaves per plant and number of ovules per flower were excluded from the analysis.

Figure 3.7. Effects of fertilizer and pollination treatments on mean (\pm SE) seed abortion in monkshood. The numbers above the plots indicate sample sizes for each treatment combination.

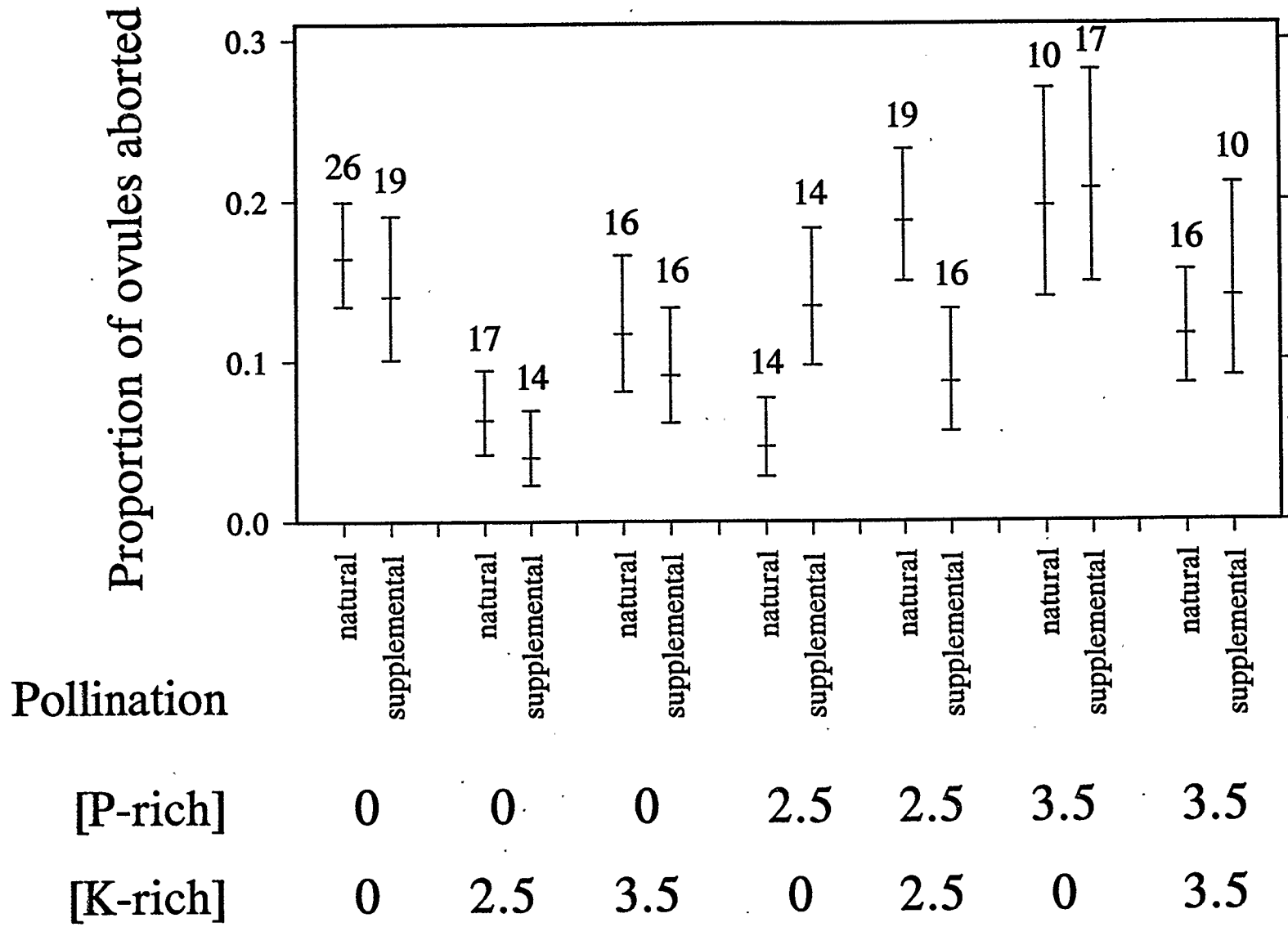


Table 3.5 Analysis of the effects of fertilization and supplemental pollination on seed abortion in monkshood.

Effects	test statistic
P-rich fertilizer	$F_{2,190} = 2.66^{**}$
K-rich fertilizer	$F_{2,190} = 5.02^{**}$
P-rich x K-rich	$F_{2,190} = 4.30^{*}$
pollination treatment	$F_{1,190} = 3.89$
P-rich x pollination	$F_{2,190} = 0.66^{**}$
K-rich x pollination	$F_{2,190} = 5.59^{*}$
P x K x pollination	$F_{2,190} = 3.27^{*}$
number of leaves	$F_{1,190} = 3.86^{*}$
inflorescence size	$F_{1,190} = 4.90$
leaves x inflorescence	$F_{1,190} = 3.12^{*}$
leaves x pollination	$F_{1,190} = 4.22^{*}$
inflorescence x K-rich	$F_{2,190} = 0.82^{*}$
inflorescence x pollination	$F_{1,190} = 4.99^{***}$
inflorescence x K x pollination	$F_{2,190} = 8.74^{***}$
leaves x inflorescence x P	$F_{2,190} = 2.07$
leaves x inflorescence x K	$F_{2,190} = 0.66$
leaves x infl x P x K	$F_{2,190} = 1.50^{*}$
leaves x infl x pollination	$F_{1,190} = 6.11^{*}$
leaves x infl x P x poll	$F_{2,190} = 1.96^{***}$
leaves x infl x K x poll	$F_{2,190} = 7.23^{***}$
leaves x infl x P x K x poll	$F_{2,190} = 5.56^{**}$

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

did not reveal any significant differences between pairs of treatment combinations. However, the probability of seed abortion ranged from a mean of 0.04 (LSE=0.023, USE=0.069) for supplementally-pollinated plants treated with a low-concentration dose of K-rich fertilizer to a mean of 0.21 (LSE=0.149, USE=0.282) for supplementally-pollinated plants treated with a high-concentration dose of P-rich fertilizer. Additionally, there was no pattern evident in the effects of supplemental pollination within fertilizer treatment combinations; supplemental pollination caused increased seed abortion in some groups but decreased it in others.

The interaction between leaf number per plant and pollination treatment (Table 3.5) was caused by the significant difference between the partial regression coefficients for the two groups ($t_{190} = -2.05$, $P < 0.05$). The partial regression coefficient for naturally-pollinated plants did not differ from zero ($b \pm s_b = -0.03 \pm 0.036$, $t_{190} = 0.08$, $P > 0.9$) whereas the relation was significantly negative for supplementally-pollinated plants ($b \pm s_b = -1.29 \pm 0.531$, $t_{190} = -2.43$, $P < 0.05$).

The interaction between inflorescence size, K-rich fertilizer and pollination treatment was partially caused by two groups with partial regression coefficients different from zero. There were negative relations between inflorescence size and abortion probability for supplementally-pollinated plants that were not fertilized ($b \pm s_b = -1.48 \pm 0.547$, $t_{190} = -2.71$, $P < 0.01$) or were treated with a moderate concentration of K-rich fertilizer ($b \pm s_b = -1.60 \pm 0.522$, $t_{190} = -3.06$, $P < 0.01$). Within each of these two fertilizer treatment groups, the relation between inflorescence size and abortion probability differs significantly between the two pollination treatments ($t_{190} = -2.63$, $P < 0.01$, and $t_{190} = -3.35$, $P < 0.001$, respectively). Within the two pollination treatments, unfertilized and moderately fertilized plants exhibited similar relations between inflorescence size and abortion probability but heavy fertilization caused the opposite relation.

The components of the five-way interaction between the two covariates and the treatments were not estimable. Therefore, I will not consider this result further.

3.4 Discussion

3.4.1 Resource and pollen limitation

The case for resource limitation of reproductive effort by larkspur seems clear: the capacity for fruit and seed production was not realized and resource enhancement increased reproductive output. In contrast, supplemental pollination failed to increase fruit or seed set. Although decreased seed and fruit set at high concentrations of fertilizer suggest that nutrient levels perhaps became toxic, resource limitation was demonstrated nonetheless.

Of the two fertilizers tested, only the P-rich fertilizer significantly affected fruit and seed production. That phosphorus should limit seed production in a resource-limited plant is not surprising because it is found in disproportionately high concentrations in seeds (Hocking 1982, Kuo *et al.* 1982). The high concentration of phosphorus in reproductive tissues implies that a deficiency will disrupt reproduction (Larcher 1975) by preventing the proper development of reproductive structures. If phosphorus was limiting in larkspur then addition of this nutrient should be reflected in increased reproductive output.

Haig and Westoby (1988a) hypothesized that resource and pollination levels should become equally limiting. This study involved combinations of pollen supplementation and resource enhancement that would increase the supply of pollen on stigmas or macronutrients available, and bring them to a point of being equally limiting of reproduction. Phosphorus enrichment increased both fruit and seed set in larkspur indicating that reproduction was limited by this nutrient in unfertilized plants. I expect that if larkspur plants were treated with increasing doses of P-rich fertilizer (but doses less than the heavy dose administered in this experiment) a point would be

reached where further fertilization would not increase fruit and seed set unless supplemental pollen were also provided. Another nutrient (perhaps potassium) might also become limiting as phosphorus levels were increased.

Unlike larkspur, monkshood is apparently not resource-limited, for although it showed the same pattern of responses to fertilization as larkspur, the effects on seed set were not statistically significant (Table 3.4). Pollen limitation is ruled out because supplemental pollination did not increase seed set. I interpret this to mean that unmanipulated monkshood is closer to being equally limited by both factors than larkspur; it appears that both resources and pollination limit seed set probability in monkshood. The fact that the combination of fertilization and supplemental pollination did not increase seed set may be due to other limitations not explored in this study. Other factors that could potentially limit reproduction include different macronutrients, micronutrients, light conditions, herbivory and seed predation. A complete assessment of all possible limitations on reproduction would involve extending the simple Haig and Westoby (1988a) model by one dimension for each additional potentially limiting factor examined. Even if other factors placed the ultimate limit on reproductive success for monkshood and larkspur in 1990, the relative importance of nutritional resources and pollen receipt for each species is clear. Additionally, these results are from a single season and reproductive limitations may shift given variability in resource and pollination levels.

Flower, seed and fruit abortion are means by which fruit and seed production can be closely matched to environmental levels (Stephenson 1981) so that the equal limitation predicted by Haig and Westoby (1988a) is achieved. The lower overall abortion rate supports the conclusion that monkshood is less resource limited than larkspur.

Other studies have shown that both resources and pollen receipt may limit reproductive output. Hainesworth *et al.* (1985) examined reproductive limitation in a generally monocarpic species, *Ipomopsis aggregata* and found that supplemental pollination significantly increased both fruit and seed set. Plants that were supplementally pollinated also showed correlations between plant size and abortion rates indicating that for these manipulated plants resources other than pollen became limiting. This study could assess the factors limiting reproduction because *I. aggregata* is typically semelparous and increased fruit or seed production due to pollination treatments would not incur a cost of reproduction that would be evident the following season. Galen *et al.* (1985) found that both pollination and maternal resource levels limited reproduction in *Clintonia borealis*; following resource manipulation, supplemental pollination and observations of flowering phenology and pollinator behavior, they concluded that seed set and seed size were a function of the balance of these two environmental factors. Harder *et al.* (1985) found that both limited pollinator service and resources for fruit development may restrict seed quality and quantity in *Erythronium americanum*; naturally-pollinated plants had a low probability of seed set implicating pollination levels, but hand-pollination resulted in low seed set possibly implicating resources although the previous chapter outlined the complicated aspects of pollination effects. Similarly, Montalvo and Ackerman (1987) concluded that both resources and pollination frequency affected fruit set in *Ionopsis utricularioides*; hand-pollination increased fruit set but subsequent patterns of fruit maturation and abortion pointed to the importance of resource availability.

One year's reproductive effort must be placed in the context of overall lifetime reproduction, however. Snow and Whigham (1989) clearly demonstrated a cost of reproduction in *Tipularia discolor*. Supplemental pollination led to greater current fruit set but reduced subsequent flowering and vegetative propagation. Conversely,

Horvitz and Schemske (1988b) manipulated reproductive effort in *Calathea ovandensis* and found no differences in survival, growth or reproduction in the following season. Studying *Ipomopsis aggregata*, Paige and Whitham (1987) found that individuals experiencing low reproductive success switched from semelparity to iteroparity. Bierzychudek (1984) found that larger individuals of *Arisaema triphyllum* were more likely to be female. This might be expected because maternal function is more expensive in terms of resources.

Furthermore, environmental conditions experienced by reproducing plants may change between years and the reproductive limitations in successive seasons may differ. A thorough examination of reproductive limitation in *Banksia spinulosa* revealed changing conditions and success in four successive years (Vaughton 1991). When natural fruit set was high, fertilization and supplemental pollination did not increase them; however, in later years low fruit set was increased by these treatments (Vaughton 1991). In contrast, pollen availability consistently limited fruit set by *Cyclopogon cranichoides* over four years (Calvo 1990).

The results of the previous chapter suggested that maternal resources might limit reproduction in larkspur and monkshood and this chapter demonstrates resource limitation for larkspur within a single season and demonstrates that in 1990 monkshood was either equally limited by pollen receipt and maternal resources or by another undetermined factor. However, repetition of the experiment over several years and monitoring of the reproductive success of individual larkspur plants between years may reveal that other factors exert long-term limitations or that several factors are equally limiting. Similarly, differing ambient levels of resources and pollination may be experienced by monkshood in successive seasons and the current balance of limitations may not persist in the longer term. Between-year variation in pollen

receipt and available resources may also explain why monkshood, though limited both by pollen and resources in 1990, nevertheless produced far more ovules than seeds.

3.4.2 Abortion of developing seeds

The immediate effect of over-initiation of seeds is that some will be aborted as a response to resource limitation. Inadequate resource levels can cause ovule abortion, as demonstrated by Harper and Wallace (1987) who found that defoliation increased ovule abortion in *Epilobium montanum*. Plants exhibiting different probabilities of seed abortion, may be responding to the relative importance of pollen and resource limitation. That is, plants with a high probability of seed abortion are more strongly influenced by resource availability than by pollen limitation, and vice versa.

Because larkspur is resource limited (see 3.4.1), resource enhancement should decrease the probability of seed abortion. The effects of treatment with P-rich fertilizer clearly support the resource-limitation of larkspur. The interaction between K-rich fertilizer treatment and pollination treatment supports the resource-limitation conclusion. Supplemental pollination exacerbated the resource limitation in unfertilized plants because more ovules were developing into seeds. As more pollen was applied while the resource pool remained constant, resource levels became even more limiting of seed set so that not all fertilized ovules could be matured. Conversely, the effect of supplemental pollination was less severe in fertilized plants. The results of the analysis of seed abortion in larkspur are not all supportive of this hypothesis, however. The partial regression coefficient for the relation between leaf number and abortion probability was more negative for supplementally-pollinated plants. The fact that heavier pollination (presumably resulting in more fertilized ovules) corresponded to lower probabilities of seed abortion, suggests that

supplemental pollination may have affected seed quality or that leaf number is not a reliable measure of resources available for seed maturation.

The complex interactions of fertilizer, pollination treatments, inflorescence size and leaf number per plant on the probability of ovule abortion in monkshood directly reflect the close balance between pollen and resource limitation in monkshood. As fertilizer and/or supplemental pollen were supplied to monkshood the limitations on seed set change and the balance shifted slightly. Several interactions were caused by particular combinations of fertilization and supplemental-pollination that resulted in low abortion probabilities or strongly negative relationships between abortion probability and a covariate.

If sufficient resources are not available to nourish all developing ovules then those offspring more likely to enhance parental fitness will be retained. Genetic quality of offspring has been previously implicated as a factor in maternal decisions (Temme 1986). In addition, multiply sired fruits in *Raphanus sativus* have greater total seed weight due to maternal influence over fertilization (Marshall and Ellstrand 1986). Regardless of the proximate causes of abortion, selective maturation of offspring will increase the average quality of those offspring (Stephenson and Winsor 1986).

These explanations propose selective abortion as the means by which parents may selectively mature offspring. In addition to this hypothesis of maturing the best prospects, Kozlowski and Stearns (1989) proposed that optimal brood size varies between reproductive episodes and that zygote over-production allows fine-tuning to this optimum. Lee and Bazzaz (1982) also implicated unpredictable resource levels in the over-initiation of fruits. Herrera (1990) ascribed abortion levels in *Lavandula latifolia* to sibling rivalry and genetic predetermination of brood size.

Abortion of fruits and seeds may represent a lost investment to the maternal plant. In *Catalpa speciosa* abortion of fruit results in lowered mass of the surviving fruit and seeds and lower germination probabilities for these seeds (Stephenson 1984). However, *Asclepias speciosa* aborted flowers and fruits before significant amounts of nutrients were invested in them (Bookman 1983).

Larkspur has large inflorescences and the total potential fecundity is large. The demonstrated resource limitation indicates that not all of this potential was utilized in the natural situation. Given this high capacity for reproduction, larkspur can employ bet-hedging strategies that allow it to take advantage of better than average conditions. In years when resources are particularly abundant more fruit and seed can be set and a fecundity benefit realized (Stephenson 1981, Lee and Bazzaz 1982, Kozlowski and Stearns 1989). In addition, hermaphroditic flowers that do not produce fruits and seeds may contribute to plant fitness paternally by siring seeds elsewhere (Willson and Rathcke 1974) or may aid in the pollination of other flowers on the same inflorescence by attracting pollinators (Stephenson 1979).

3.4.3 Seed size and seed number

If maternal function is resource limited then trade-offs will occur between the competing reproductive costs drawing on the resource pool (Haig and Westoby 1988b). Allocation of resources to seed development is one such cost and seed size and number trade-offs will reflect limitation of resources. A negative relation between seed number and seed size would be evidence of a trade-off based on allocation of limited resources. In contrast, although larkspur was resource-limited in terms of the probability of setting seed, seed mass varied positively with the number of seeds produced per flower. Monkshood also exhibited a positive relationship between seed mass and seed number per flower.

Venable (1992) offered several explanations for positive relations between seed size and number. First, plant size, which is a measure of the resource pool, is more variable than seed size and this variation will tend to mask the seed number-seed size trade-off. Second, the model of Smith and Fretwell (1974), which predicted that there is an optimal seed size that maximizes parental fitness and that there will be little within-brood variation in seed size, assumes linearly increasing parental fitness with seed number. However, Venable (1992) assumes a non-homogeneous function which means that the fitness-maximizing seed size will vary with resource availability. An example of such a non-homogeneous fitness function is the tendency for seeds to be dispersed close to the maternal parent (Venable 1992); the optimal seed size is larger for seeds germinating close to the parent plant where they will experience greater competition.

Another explanation is that seed size and number trade-offs may be more limited by developmental or physiological constraints at a scale smaller than the entire plant. Plant modules often function as largely separate physiological units (Watson and Casper 1984) and investment decisions should be made at the level of those investment units rather than at the scale of the entire plant. Lloyd (1980) hypothesized that decisions regarding maturation of flowers, seeds and fruit are made at the level of these units based on maternal resource status. Similarly, Herrera (1991) suggested that inflorescences of *Lavandula stoechas* operated as separate modules and that resources could not be re-distributed among them. This varies from the more simplistic view of Smith and Fretwell (1974) where the tradeoff operated at the scale of the whole organism rather than separate modules.

3.4.4 Plant vigor

Several results suggest that more than just resources and pollination levels affect reproductive success in larkspur. Other measures of plant size, and therefore of

vigor and available resources, are correlated with the probability of seed set. More vigorous plants have superior genotypes and will survive and reproduce more successfully under given environmental conditions than less vigorous plants. For example, the chance of an ovule setting seed was positively related to the number of leaves per plant. This suggests that more vigorous plants can allocate more resources to both vegetative and reproductive processes and that the larger reproductive allocation means more seeds can be produced. For larkspur the conclusion that resource limitation and plant vigor are the most important factors affecting reproductive success is further supported by the result that mean seed weight was positively correlated with both seed number and ovule number per flower.

Seed set probabilities in larkspur were positively related to the number of leaves per plant and the number of ovules per flower. The interactions between these covariates and the fertilization effects were not significant, indicating that resource limitation was independent of measures of plant vigor. Both vigorous and less vigorous plants were equally affected by resource limitation of seed set. However, the greater seed set in more vigorous larkspur was apparently due to their ability to make more efficient use of the resources they did have. Conversely, Dudash (1991) found that seed mass per fruit in *Sabatia angularis* varied between seasons but was unaffected by plant size. Seed mass per fruit in large and small plants was equally affected by ecological factors (Dudash 1991) and apparently there was no difference in the ability of different individuals to utilize available resources.

The same effect was determined for monkshood. Seed size increased with the mean number of seeds per flower but decreased with increasing inflorescence size. I interpret these apparently conflicting results to mean that monkshood is closer to the balance point of resource allocation described by Haig and Westoby (1988a).

4. Conclusions

Larkspur and monkshood are self-compatible but predominantly outcrossing species. However, larkspur experiences inbreeding depression reflected in reduced seed mass following self-fertilization. Overlap in the flowering phenologies of larkspur and monkshood did not coincide with reduced reproductive success as might be expected if pollinators exhibited switching behavior that produced interspecific pollinations at the expense of intraspecific pollination frequency. Competition for pollination is not a limiting factor in the reproduction of larkspur and monkshood. Diversity of pollen donors is also not a limiting factor because pollinations with different numbers of donors did not affect reproductive success.

Simultaneous resource enhancement and supplemental pollination of larkspur and monkshood showed that larkspur was limited by phosphorus availability. On the other hand, monkshood was equally limited by both pollen receipt and nutrient levels. Patterns of seed abortion corroborate the conclusion that resources primarily were limiting for larkspur but for monkshood they were limiting in conjunction with pollination levels. Seed abortion may also be a mechanism by which monkshood achieved equal limitation by resources and pollen. Many studies have shown particular plant species to be either resource- or pollen-limited, but relatively few consider the interaction between potentially limiting factors. Similarly, abortion of seeds and fruits is widely documented, but has not been placed in the context of limitations on reproduction.

Ambient resource levels and pollinator availabilities may change from year to year, and limiting factors may differ between seasons. Long-term limitations of reproductive success may be more important in determining selective pressures on reproductive characteristics of larkspur and monkshood. Additionally, high fecundity in a single season may result in lowered reproductive effort in the subsequent season

but this will be subject to the particular conditions of the subsequent season. Future studies of larkspur and monkshood should monitor individual plants in successive seasons so that long-term reproductive limitations and costs of reproduction can be determined. Some studies that have involved examination of reproductive success between years have demonstrated costs of reproduction for plants. Examination of reproduction in perennial species must involve within-year assessments of reproductive limitations placed in the context of lifetime reproductive success.

Plant vigor is an important influence on reproductive success in larkspur and monkshood. Analysis of reproductive success revealed that plant size was often involved in determining levels of seeds set and affected seed size and number trade-offs. Future studies of within-year limitations on larkspur, monkshood and other species should also endeavor to separate the external effects of pollination and resource availability on reproductive success from those inherent to the individual plant. Furthermore, determining the influence of plant vigor on costs of reproduction may be difficult, but would also be instructive in understanding reproductive limitations in plants.

5. Literature cited

- Ackerman, J.D. 1989. Limitations to sexual reproduction in *Encyclia krugii* (Orchidaceae). *Systematic Botany* 14:101-109.
- Ackerman, J.D. and A.M. Montalvo. 1990. Short- and long-term limitations to fruit production in a tropical orchid. *Ecology* 71:263-272.
- Aker, C.L. 1982. Spatial and temporal dispersion patterns of pollinators and their relationship to the flowering strategy of *Yucca whipplei* (Agavaceae). *Oecologia* 54:243-252.
- Allen, R.B. and J.B. Wilson. 1992. Fruit and seed production in *Berberis darwinii* Hook., a shrub recently naturalised in New Zealand. *New Zealand Journal of Botany* 30:45-55.
- Arnold, R.M. 1982. Pollination, predation and seed set in *Linaria vulgaris* (Scrophulariaceae). *The American Midland Naturalist* 107:360-369.
- Barrett, S.C.H. and D. Charlesworth. 1991. Effects of a change in the level of inbreeding on the genetic load. *Nature* 352:522-524.
- Bawa, K.S. and J.H. Beach. 1981. Evolution of sexual systems in flowering plants. *Annals of the Missouri Botanical Garden* 68:154-174.
- Bawa, K.S. and C.J. Webb. 1984. Flower, fruit and seed abortion in tropical forest trees: Implications for the evolution of paternal and maternal reproductive patterns. *American Journal of Botany* 71:736-751.
- Becerra, J.X. and D.G. Lloyd. 1992. Competition-dependent abscission of self-pollinated flowers of *Phormium tenax* (Agavaceae): A second action of self-incompatibility at the whole flower level. *Evolution* 46: 458-469.
- Berry, P.E. and R.N. Calvo. 1991. Pollinator limitation and position dependent fruit set in the high Andean orchid *Myrosmodes cochleare* (Orchidaceae). *Plant Systematics and Evolution* 174:93-101.

- Bertin, R.I. 1982a. Paternity and fruit production in trumpet creeper (*Campsis radicans*). The American Naturalist 119:694-709.
- _____. 1982b. Floral biology, hummingbird pollination and fruit production of trumpet creeper (*Campsis radicans*, Bignoniaceae). American Journal of Botany 69:122-134.
- _____. 1990. Effects of pollination intensity in *Campsis radicans*. American Journal of Botany 77:178-187.
- Bierzychudek, P. 1981. Pollinator limitation of plant reproductive effort. The American Naturalist 117:838-840.
- _____. 1984. Determinants of gender in Jack-in-the-pulpit: The influence of plant size and reproductive history. Oecologia 65:14-18.
- Bloom, A.J., F.S. Chapin and H.A. Mooney. 1985. Resource limitation in plants - An economic analogy. Annual Review of Ecology and Systematics 16:363-392.
- Bookman, S.S. 1983. Costs and benefits of flower abscission and fruit abortion in *Asclepias speciosa*. Ecology 64:264-273.
- _____. 1984. Evidence for selective fruit production in *Asclepias*. Evolution 38:72-86.
- Brink, D.E. 1980. Reproduction and variation in *Aconitum columbianum* (Ranunculaceae), with emphasis on California populations. American Journal of Botany 67:263-273.
- Bronstein, J.L. 1988. Limits to fruit production in a monoecious fig: Consequences of an obligate mutualism. Ecology 69:207-214.
- Calvo, R.N. 1990. Four-year growth and reproduction of *Cyclopogon cranichoides* (Orchidaceae) in South Florida. American Journal of Botany 77:736-741.

- Campbell, D.R. 1985. Pollinator sharing and seed set of *Stellaria pubera*: Competition for pollination. *Ecology* 66:544-553.
- _____. 1987. Interpopulational variation in fruit production: The role of pollination-limitation in the Olympic Mountains. *American Journal of Botany* 74:269-273.
- _____. 1991. Effects of floral traits on sequential components of fitness in *Ipomopsis aggregata*. *The American Naturalist* 137:713-737.
- Campbell, D.R. and A.F. Motten. 1985. The mechanism of competition for pollination between two forest herbs. *Ecology* 66:554-563.
- Charlesworth, D. and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* 18:237-268.
- Clegg, M.T. 1980. Measuring plant mating systems. *Bioscience* 30:814-818.
- Darwin, C. 1876. On the Effects of Cross and Self Fertilisation in the Vegetable Kingdom. John Murray, London.
- Delph, L.F. 1986. Factors regulating fruit and seed production in the desert annual *Lesquerella gordonii*. *Oecologia* 69:471-476.
- de Nettancourt, D. 1977. Incompatibility in Angiosperms. Springer-Verlag, New York.
- Dudash, M.R. 1991. Plant size effects on female and male function in hermaphroditic *Sabatia angularis* (Gentianaceae). *Ecology* 72:1004-1012.
- Eckert, C.G. and S.C.H. Barrett. 1992. Stochastic loss of style morphs from populations of tristylous *Lythrum salicaria* and *Decodon verticillatus* (Lythraceae). *Evolution* 46:1014-1029.
- Ehrlén, J. 1992. Proximate limits to seed production in a herbaceous perennial legume, *Lathyrus vernus*. *Ecology* 73:1820-1831.

- Ellstrand, N.C. 1984. Multiple paternity within fruits of the wild radish, *Raphanus sativus*. *The American Naturalist* 123:819-828.
- Ellstrand, N.C., A.M. Torres and D.A. Levin. 1978. Density and rate of apparent outcrossing in *Helianthus annuus* (Asteraceae). *Systematic Botany* 3:403-407.
- Faegri, K. and J. Iverson. 1989. Textbook of pollen analysis. 4th ed. John Wiley & Sons Ltd., Chichester, England.
- Feinsinger, P. and H.M. Tiebout III. 1991. Competition among plants sharing hummingbird pollinators: Laboratory experiments on a mechanism. *Ecology* 72:1946-1952.
- Fenster, C.B. 1991. Effect of male pollen donor and female seed parent on allocation of resources to developing seeds and fruit in *Chamaecrista fasciculata* (Leguminosae). *American Journal of Botany* 78:13-23.
- Fox, J.F. 1992. Pollen limitation of reproductive effort in willows. *Oecologia* 90:283-287.
- Free, J.B. 1968. Dandelion as a competitor to fruit trees for bee visits. *Journal of Applied Ecology* 56:169-178.
- Galen, C. and T. Gregory. 1989. Interspecific pollen transfer as a mechanism of competition: Consequences of foreign pollen contamination for seed set in the alpine wildflower, *Polemonium viscosum*. *Oecologia* 81:120-123.
- Galen, C. and H.G. Weger. 1986. Re-evaluating the significance of correlations between seed number and size: Evidence from a natural population of the lily, *Clintonia borealis*. *American Journal of Botany* 73:346-352.
- Galen, C., R.C. Plowright and J.D. Thomson. 1985. Floral biology and regulation of seed set and seed size in the lily, *Clintonia borealis*. *American Journal of Botany* 72:1544-1552.

- Garwood, N.C. and C.C. Horvitz. 1985. Factors limiting fruit and seed production of a temperate shrub, *Staphylea trifolia* L. (Staphyleaceae). *American Journal of Botany* 72:453-466.
- Gorchov, D.L. 1988. Effects of pollen and resources on seed number and other fitness components in *Amelanchier arborea* (Rosaceae: Maloideae). *American Journal of Botany* 75:1275-1285.
- Gross, R.S. and P.A. Werner. 1983. Relationships among flowering phenology, insect visitors, and seed-set of individuals: Experimental studies on four co-occurring species of goldenrod (*Solidago*: Compositae). *Ecological Monographs* 53:95-117.
- Haig, D. 1990. Brood reduction and optimal parental investment when offspring differ in quality. *The American Naturalist* 136:550-556.
- Haig, D. and M. Westoby. 1988a. On limits to seed production. *The American Naturalist* 131:757-759.
- _____. 1988b. Inclusive fitness, seed resources, and maternal care. Pages 60-79 in J. Lovett Doust and L. Lovett Doust, editors. *Plant Reproductive Ecology*. Oxford University Press, New York.
- Hainesworth, F.R., L.L. Wolf and T. Mercier. 1985. Pollen limitation in a monocarpic species, *Ipomopsis aggregata*. *Journal of Ecology* 73:263-270.
- Harder, L.D., J.D. Thomson, M.B. Cruzan and R.S. Unnasch. 1985. Sexual reproduction and variation in floral morphology in an ephemeral vernal lily, *Erythronium americanum*. *Oecologia* 67:286-291.
- Harding, J., C.B. Mankinen and M.H. Elliot. 1974. Genetics of *Lupinus*. VII. Outcrossing, autofertility, and variability in natural populations of the *nanus* group. *Taxon* 23:729-738.

- Harper, J.L. and H.L. Wallace. 1987. Control of fecundity through abortion in *Epilobium montanum* L. *Oecologia* 74:31-38.
- Hebert, P.D.N. and M.J. Beaton. 1989. Methodologies for Allozyme Analysis Using Cellulose Acetate Electrophoresis. Helena Laboratories, Beaumont, Texas.
- Herrera, C.M. 1990. Brood size reduction in *Lavandula latifolia* (Labiatae): A test of alternative hypotheses. *Evolutionary Trends in Plants* 4:99-105.
- Herrera, J. 1991. Allocation of reproductive resources within and among inflorescences of *Lavandula stoechas* (Lamiaceae). *American Journal of Botany* 78:789-794.
- Hocking, P.J. 1982. The nutrition of fruits of two proteaceous shrubs, *Grevilla wilsonii* and *Hakea undulata*, from south-western Australia. *Australian Journal of Botany* 30:219-230.
- Horvitz, C.C. and D.W. Schemske. 1988a. A test of the pollinator limitation hypothesis for a neotropical herb. *Ecology* 69:200-206.
- _____. 1988b. Demographic cost of reproduction in a neotropical herb: An experimental field study. *Ecology* 69:1741-1745.
- Hultén, E. 1968. Flora of Alaska and Neighboring Territories. A Manual of the Vascular Plants. Stanford University Press, Stanford, California.
- Jain, S.K. 1976. The evolution of inbreeding in plants. *Annual Review of Ecology and Systematics* 7:469-495.
- Janzen, D.H. 1977. A note on optimal mate selection in plants. *The American Naturalist* 111:365-371.
- Janzen, D.H., P. DeVries, D.E. Gladstone, M.L. Higgins and T.M. Lewinsohn. 1980. Self- and cross-pollination of *Encyclia cordigera* (Orchidaceae) in Santa Rosa National Park, Costa Rica. *Biotropica* 12:72-74.

- Johnston, M.O. 1991a. Natural selection on floral traits in two species of *Lobelia* with different pollinators. *Evolution* 45:1468-1479.
- _____. 1991b. Pollen limitation of female reproduction in *Lobelia cardinalis* and *L. siphilitica*. *Ecology* 72:1500-1503.
- Karoly, K. 1992. Pollinator limitation in the facultatively autogamous annual, *Lupinus nanus* (Leguminosae). *American Journal of Botany* 79:49-56.
- Kozlowski, J. and S.C. Stearns. 1989. Hypotheses for the production of excess zygotes: Models of bet-hedging and selective abortion. *Evolution* 43:1369-1377.
- Kress, W.J. 1981. Sibling competition and evolution of pollen unit, ovule number, and pollen vector in angiosperms. *Systematic Botany* 6:101-112.
- Kuo, J., P.J. Hocking and J.S. Pate. 1982. Nutrient reserves in seeds of selected proteaceous species from south-western Australia. *Australian Journal of Botany* 30:231-249.
- Kwak, M.M. and O. Jennersten. 1986. The significance of pollination time and frequency and of purity of pollen loads for seed set in *Rhinanthus angustifolius* (Scrophulariaceae) and *Viscaria vulgaris* (Caryophyllaceae). *Oecologia* 70:502-507.
- _____. 1991. Bumblebee visitation and seedset in *Melampyrum pratense* and *Viscaria vulgaris*: heterospecific pollen and pollinator limitation. *Oecologia* 86:99-104.
- LaLonde, R.G. and B.D. Roitberg. 1989. Resource limitation and offspring size and number trade-offs in *Cirsium arvense* (Asteraceae). *American Journal of Botany* 76:1107-1113.
- Lande, R. and D.W. Schemske. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* 39:24-40.
- Larcher, W. 1975. *Physiological Plant Ecology*. Springer Verlag. Berlin.

- Lee, T.D. 1984. Patterns of fruit maturation: A gametophyte competition hypothesis. *The American Naturalist* 123:427-432.
- _____. 1988. Patterns of fruit and seed production. Pages 179-202 in J. Lovett Doust and L. Lovett Doust, editors. *Plant Reproductive Ecology*. Oxford University Press, New York.
- Lee, T.D. and F.A. Bazzaz. 1980. Effects of defoliation and competition in the annual plant *Abutilon theophrasti*. *Journal of Ecology* 68:813-821.
- _____. 1982. Regulation of fruit and seed production in an annual legume, *Cassia fasciculata*. *Ecology* 63:1363-1373.
- Levin, D.A. 1988. The paternity pools of plants. *The American Naturalist* 132:309-317.
- _____. 1989. Inbreeding depression in partially self-fertilizing *Phlox*. *Evolution* 43:1417-1423.
- Levin, D.A. and W.W. Anderson. 1970. Competition for pollinators between simultaneously flowering species. *The American Naturalist* 104:455-467.
- Levin, D.A. and H.W. Kerster. 1974. Gene flow in seed plants. *Evolutionary Biology* 7:139-220.
- Lloyd, D.G. 1979. Some reproductive factors affecting the selection of self fertilization in plants. *The American Naturalist* 113:67-79.
- _____. 1980. Sexual strategies in plants. I. An hypothesis of serial adjustments of maternal investment during one reproductive session. *New Phytologist* 86:69-79.
- Lloyd, D.G. and C.J. Webb. 1986. The avoidance of interference between the presentation of pollen and stigmas in angiosperms. I. Dichogamy. *New Zealand Journal of Botany* 24:135-162.

- Lloyd, D.G., C.J. Webb and R.B. Primack. 1980. Sexual strategies in plants.
II. Data on the temporal regulation of maternal investment. *New Phytologist* 86:81-92.
- Lubbers, A.E. and N.L. Christensen. 1986. Intraseasonal variation in seed production among flowers and plants of *Thalictrum thalictroides* (Ranunculaceae). *American Journal of Botany* 73:190-203.
- Manasse, R.S. and K. Pinney. 1991. Limits to reproductive success in a partially self-incompatible herb: Fecundity depression at serial life-cycle stages. *Evolution* 45:712-720.
- Marshall, D.R. 1991. Nonrandom mating in wild radish: Variation in pollen donor success and effects of multiple paternity among one- to six-donor pollinations. *American Journal of Botany* 78:1404-1418.
- Marshall, D.R. and N.C. Ellstrand. 1986. Sexual selection in *Raphanus sativus*: Experimental data on nonrandom fertilization, maternal choice, and consequences of multiple paternity. *The American Naturalist* 127:446-461.
- _____. 1988. Effective mate choice in wild radish: Evidence for selective seed abortion and its mechanism. *The American Naturalist* 131: 739-756.
- Maun, M.A. and P.B. Cavers. 1971. Seed production and dormancy in *Rumex crispus*. 1. The effects of removal of cauline leaves at anthesis. *Canadian Journal of Botany* 49:1123-1130.
- McCall, C. and R.B. Primack. 1985. Effects of pollen and nitrogen availability on reproduction in a woodland herb, *Lysimachia quadrifolia*. *Oecologia* 67:403-410.
- McDade, L.A. and P. Davidar. 1984. Determinants of fruit and seed set in *Pavonia daspetala* (Malvaceae). *Oecologia* 64:61-67.

- Michaels, H.J., B. Benner, A.P. Hartgerink, T.D. Lee, S. Rice, M.F. Willson and R.I. Bertin. 1988. Seed size variation: Magnitude, distribution, and ecological correlates. *Evolutionary Ecology* 2:157-166.
- Milliken, G.A. and D.E. Johnson. 1984. *Analysis of Messy Data*. vol.1. *Designed Experiments*. Van Nostrand Reinhold, New York.
- Montalvo, A.M. and J.D. Ackerman. 1987. Limitations to fruit production in *Ionopsis utricularioides* (Orchidaceae). *Biotropica* 19:24-31.
- Morse, D.H. and R.S. Fritz. 1983. Contributions of diurnal and nocturnal insects to the pollination of common milkweed (*Asclepias syriaca* L.) in a pollen limited system. *Oecologia* 60:190-197.
- Moss, E.H. 1983. *Flora of Alberta*. 2nd ed. revised by J.G. Packer. University of Toronto Press, Toronto.
- Mulcahy, D.L. 1974. Correlation between speed of pollen tube growth and seedling height in *Zea mays* L. *Nature* 249:491-493.
- _____. 1979. The rise of angiosperms: A genecological factor. *Science* 206:20-23.
- Neter, J., W. Wasserman and M.H. Kutner. 1985. *Applied Linear Statistical Models*. Regression, Analysis of Variance and Experimental Designs. 2nd ed. Richard D. Irwin, Inc., Homewood, Illinois.
- Paige, K.N. and T.G. Whitham. 1987. Flexible life history traits: Shifts by scarlet gilia in response to pollinator abundance. *Ecology* 68:1691-1695.
- Petersen, C., J.H. Brown and A. Kodric-Brown. 1982. An experimental study of floral display and fruit set in *Chilopsis linearis* (Bignoniaceae). *Oecologia* 55:7-11.
- Porsild, A.E. and W.J. Cody. 1980. *Vascular Plants of Continental Northwest Territories, Canada*. National Museum of Natural Sciences, National Museums of Canada.

- Queller, D.C. 1983. Kin selection and conflict in seed maturation. *Journal of Theoretical Biology* 100:153-172.
- _____. 1985. Proximate and ultimate causes of low fruit production in *Asclepias exaltata*. *Oikos* 44:373-381.
- Rathcke, B. 1983. Competition and facilitation among plants for pollination. Pages 305-329 in L. Real, editor. *Pollination Biology*. Academic Press, Orlando, Florida.
- Richards, A.J. 1986. *Plant Breeding Systems*. George Allen & Unwin, London.
- Ridley, L. 1988. A preliminary palynological study of the Ranunculaceae tribe Helleboreae native to Alberta. Unpublished manuscript.
- Ritland, K. 1983. Estimation of mating systems. Pages 289-302 in S.D. Tanksley and T.J. Orton, editors. *Isozymes in Plant Genetics and Breeding, Part A*. Elsevier Science Publishers B.V., Amsterdam.
- Ritland, K. and S. Jain. 1981. A model for the estimation of outcrossing rate and gene frequencies using n independent loci. *Heredity* 47:35-52.
- SAS. 1987. *SAS/STAT Guide for Personal Computers, Version 6 Edition*. SAS Institute Inc., Cary, North Carolina.
- Schemske, D.W. and R. Lande. 1985. The evolution of self-fertilization and inbreeding depression in plants. II. Empirical observations. *Evolution* 39:41-52.
- Schoen, D.J. 1982. The breeding system of *Gilia achilleifolia*: variation in floral characteristics and outcrossing rate. *Evolution* 36:352-360.
- Smith, C.C. and S.D. Fretwell. 1974. The optimal balance between size and number of offspring. *The American Naturalist* 108:499-506.
- Snow, A.A. 1982. Pollination intensity and potential seed set in *Passiflora vitifolia*. *Oecologia* 55:231-237.

- _____. 1986. Pollination dynamics in *Epilobium canum* (Onagraceae): Consequences for gametophytic selection. *American Journal of Botany* 73:139-151.
- _____. 1990. Effects of pollen-load size and number of donors on sporophyte fitness in wild radish (*Raphanus raphanistrum*). *The American Naturalist* 136:742-758.
- Snow, A.A. and T.P. Spira. 1991. Pollen vigour and the potential for sexual selection in plants. *Nature* 352:796-797.
- Snow, A.A. and D.F. Whigham. 1989. Costs of flower and fruit production in *Tipularia discolor* (Orchidaceae). *Ecology* 70:1286-1293.
- Sork, V.L. and D.W. Schemske. 1992. Fitness consequences of mixed-donor pollen loads in the annual legume *Chamaecrista fasciculata*. *American Journal of Botany* 79:508-515.
- Stephenson, A.G. 1979. An evolutionary examination of the floral display of *Catalpa speciosa* (Bignoniaceae). *Evolution* 33:1200-1209.
- _____. 1980. Fruit set, herbivory, fruit reduction, and the fruiting strategy of *Catalpa speciosa* (Bignoniaceae). *Ecology* 61:57-64.
- _____. 1981. Flower and fruit abortion: Proximate causes and ultimate functions. *Annual Review of Ecology and Systematics* 12:253-279.
- _____. 1984. The cost of over-initiating fruit. *The American Midland Naturalist* 112:379-386.
- Stephenson, A.G. and R.I. Bertin. 1983. Male competition, female choice, and sexual selection in plants. Pages 109-149 in L. Real, editor. *Pollination Biology*. Academic Press, Orlando, Florida.
- Stephenson, A.G. and J.A. Winsor. 1986. *Lotus corniculatus* regulates offspring quality through selective fruit abortion. *Evolution* 40:453-458.

- Sutherland, S. 1986. Floral sex ratio, fruit-set, and resource allocation in plants. *Ecology* 67:991-1001.
- Sutherland, S. and L.F. Delph. 1984. On the importance of male fitness in plants: Patterns of fruit-set. *Ecology* 65:1093-1104.
- Temme, D.H. 1986. Seed size variability: A consequence of variable genetic quality among offspring? *Evolution* 40:414-417.
- Thomson, J.D. and R.C. Plowright. 1980. Pollen carryover, nectar rewards, and pollinator behavior with special reference to *Diervilla lonicera*. *Oecologia* 46:68-74.
- Thomson, J.D. and B.A. Thomson. 1989. Dispersal of *Erythronium grandiflorum* pollen by bumblebees: Implications for gene flow and reproductive success. *Evolution* 43:657-661.
- Thomson, J.D., B.J. Andrews and R.C. Plowright. 1981. The effect of a foreign pollen on ovule development in *Diervilla lonicera* (Caprifoliaceae). *New Phytologist* 90:777-783.
- Travis, J. 1984. Breeding system, pollination, and pollinator limitation in a perennial herb, *Amianthus muscaetoxicum* (Liliaceae). *American Journal of Botany* 71:941-947.
- Udovic, D. 1981. Determinants of fruit set in *Yucca whipplei*: Reproductive expenditure vs. pollinator availability. *Oecologia* 48:389-399.
- Van Steveninck, R.F.M. 1957. Factors affecting the abscission of reproductive organs in yellow lupines (*Lupinus luteus*). I. The effect of different patterns of flower removal. *Journal of Experimental Botany* 8:373-381.
- Vaughton, G. 1991. Variation between years in pollen and nutrient limitation of fruit-set in *Banksia spinulosa*. *Journal of Ecology* 78:389-400.

- Venable, D.L. 1992. Size-number trade-offs and the variation of seed size with plant resource status. *The American Naturalist* 140:287-304.
- Waser, N.M. 1978a. Interspecific pollen transfer and competition between co-occurring plant species. *Oecologia* 36:223-236.
- _____. 1978b. Competition for hummingbird pollination and sequential flowering in two Colorado wildflowers. *Ecology* 59:934-944.
- _____. 1983. Competition for pollination and floral character differences among sympatric plant species: A review of evidence. Pages 277-293 in C.E. Jones and R.J. Little, editors. *Handbook of Experimental Pollination Biology*. Van Nostrand Reinhold Company Inc., New York, New York.
- _____. 1986. Flower constancy: Definition, cause, and measurement. *The American Naturalist* 127:593-603.
- Waser, N.M. and M.L. Fugate. 1986. Pollen precedence and stigma closure: A mechanism for competition for pollination between *Delphinium nelsonii* and *Ipomopsis aggregata*. *Oecologia* 70:573-577.
- Waser, N.M. and L.A. Real. 1979. Effective mutualism between sequentially flowering plant species. *Nature* 281:670-672.
- Watson, M.A. and B.B. Casper. 1984. Morphogenetic constraints on patterns of carbon distribution in plants. *Annual Review of Ecology and Systematics* 15:233-258.
- Wendel, J.F. and N.F. Weeden. 1989. Visualization and Interpretation of Plant Isozymes. Pages 5-45 in *Isozymes in Plant Biology*. D.E. Soltis and P.S. Soltis, eds. *Advances in Plant Sciences Series*, vol.4. Dioscorides Press, Portland, Oregon.

- Whelan, R.J. and R.L. Goldingay. 1989. Factors affecting fruit-set in *Telopea speciosissima* (Proteaceae): The importance of pollen limitation. *Journal of Ecology* 77:1123-1134.
- Wiens, D., D.L. Nickrent, C.I. Davern, C.L. Calvin and N.J. Vivrette. 1989. Developmental failure and loss of reproductive capacity in the rare palaeoendemic shrub *Dedeckera eurekaensis*. *Nature* 338:65-67.
- Willson, M.F. 1979. Sexual selection in plants. *The American Naturalist* 113:777-790.
- Willson M.F. and N. Burley. 1983. *Mate Choice in Plants*. Princeton University Press, Princeton, New Jersey.
- Willson, M.F. and P.W. Price. 1980. Resource limitation of fruit and seed production in some *Asclepias* species. *Canadian Journal of Botany* 58:2229-2233.
- Willson, M.F. and B.J. Rathcke. 1974. Adaptive design of floral display in *Asclepias syriaca* L. *The American Midland Naturalist* 92:47-57.
- Winsor, J.A., L.E. Davis and A.G. Stephenson. 1987. The relationship between pollen load and fruit maturation and the effect of pollen load on offspring vigor in *Cucurbita pepo*. *The American Naturalist* 129:643-656.
- Wulff, R.D. 1986. Seed size variation in *Desmodium paniculatum*. II. Effects on seedling growth and physiological performance. *Journal of Ecology* 74:99-114.
- Zimmerman, J.K. and T.M. Aide. 1989. Patterns of fruit production in a neotropical orchid: Pollinator vs. resource limitation. *American Journal of Botany* 76:67-73.
- Zimmerman, M. and G.H. Pyke. 1988. Reproduction in *Polemonium*: Assessing the factors limiting seed set. *The American Naturalist* 131:723-738.