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The Incidence and Functional Significance  
of Nectarless Flowers

by

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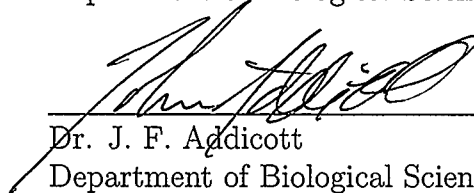
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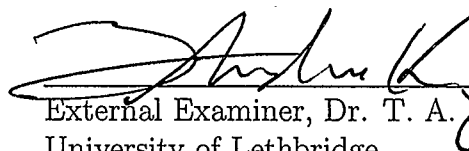
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## Abstract

Although rarely reported in the literature on plant reproduction, nectarless flowers occurred in 81% of the 52 species that I examined, suggesting that they occur commonly in natural populations. The proportion of empty flowers varied positively among species with flower size, indicating that they may save energy for small-flowered species. In contrast, a negative effect of the number of open flowers indicated that empty flowers may manipulate pollinators in species with large floral displays. Results from two field experiments concerning the effects of empty flowers on pollinator behaviour and pollen transfer also support the pollination-enhancement hypothesis. Nectar removal from differing proportions of flowers decreased the number of flowers visited by bees and the time they spent on each flower, increasing the probability of a pollinator's departure from a plant. An increased proportion of empty flowers also increased pollen removal and pollen export, but decreased self-pollination for an orchid species.

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# Chapter 1

## Nectar, Empty Flowers and Objectives

“Apparently, plants are able to attract pollinators while at the same time encouraging them to leave quickly.”

*de Jong et al.* (1993, page 325)

### 1.1 Floral Nectar and Plant Reproduction

Being immobile, outcrossing plants must rely on pollen vectors to disperse their pollen, so that they can fertilize ovules on other plants and receive pollen from other individuals. Most plants attract animals with large colourful displays and fragrances (Eriksson and Bremer, 1992), and most of these species use nectar as a food source for their pollinators to encourage visits to flowers and ensure pollen import and export (Simpson and Neff, 1983; Ackerman et al., 1994)

Floral nectar is an intricate and dynamic mechanism for promoting cross-pollination. Nectar replenishment encourages pollinators to revisit a plant

in the future, promoting more opportunities for pollination, and the secretion rate influences the volume of nectar that accumulates between visits. Thus, by adjusting nectar amounts, plants may control the number of flowers a pollinator visits and the time it spends on each flower (Thomson and Plowright, 1980; Hodges, 1981; Harder and Thomson, 1989). The number of visits that plants receive is the single most important factor influencing their pollen import and export (Nakamura et al., 1989; Young and Stanton, 1990; Kudo and Harder, 2005). In addition, visit duration positively influences opportunities for pollen exchange between flowers and pollinators (Harder and Thomson, 1989; Cresswell, 1999). However, increased pollen removal by individual pollinators can also increase within-flower self-pollination (autogamy: Rathcke and Real, 1993; Eckert, 2000) and pollen wastage (Harder and Wilson, 1998; Harder et al., 2001). Nectar availability can also influence the opportunity for between-flower self-pollination (geitonogamy), because individual pollinators visit more flowers after encountering abundant nectar (Thomson and Plowright, 1980; Hodges and Wolf, 1981). Both autogamy and geitonogamy can reduce the amount of pollen available for export (pollen discounting: Harder and Barrett, 1995) and for self-compatible species they increase self-fertilization and the exposure of offspring to inbreeding depression (Charlesworth and Charlesworth, 1987a; Husband and Schemske, 1996). Thus, floral nectar production may be under opposing selection to enhance pollinator attraction, while limiting self-pollination and pollen loss (Harder and Barrett, 1995; Harder and Wilson, 1998).

In this thesis I consider the influences on within-plant variation in nectar production and its consequences for plant reproduction. Before introducing this topic, I review relevant features of nectar production, including the composition and secretion of nectar, environmental influences, pollinator interactions and hypotheses concerning nectar secretion as a reproductive strategy.

## 1.2 Composition and Production of Nectar

Nectar is composed of water, sugars and several secondary constituents, including amino acids, lipids antioxidants and alkaloids (Baker and Baker, 1975). A cluster of secretory cells, known as the nectary, delivers nectar to the outside environment. Nectaries occur on both floral or extra-floral tissues (Alder, 2000); extra-floral nectaries reward animals (typically ants) that defend a plant from herbivores (Heads and Lawton, 1985), whereas most nectaries are floral and function in pollination.

Floral nectar plays a predominant role in plant-pollinator interactions and their evolution (Baker and Baker, 1973; Baker, 1975; Baker and Baker, 1975). Nectar production and composition vary with pollinator type: plants pollinated by high-energy pollinators (hummingbirds, hawk moths, bats) produce more nectar per flower than those pollinated by pollinators that require less energy (bees, butterflies, flies; Cruden et al., 1983). Pyke and Waser's (1981) survey of 202 hummingbird-pollinated species and 156 bee-pollinated species found that bird-pollinated flowers have dilute nectar (23% sugar content) relative to bee-pollinated flowers (36% sugar content). Pollinator mechanics involved in foraging may explain composition differences (Harder, 1983, 1986), as nectar viscosity increases exponentially with concentration, so that concentrated nectar is too viscous for efficient extraction (Pyke and Waser, 1981). Bolten and Feinsinger (1978) suggested that specific nectar compositions evolve to deter less effective pollinator types. In addition, plants pollinated by diurnal pollinators often secrete nectar only during day, whereas those pollinated by nocturnal pollinators commonly produce nectar at dusk and during night (Cruden et al., 1983). Concurrence of nectar secretion with pollinator activity minimizes the chance of nectar consumption by illegitimate pollinators.

Cruden et al. (1983) categorized species into three classes based on their nectar production patterns: 1) slow producers secrete 5-10% of their maximum accumulation per hour, 2) fast producers accumulate 22-68% of their maximum per hour and 3) super producers secrete the amount of nectar of fast producers during 2-3 hours. Slow producers often have a thick corolla or calyx that provides more protection for nectar from illegitimate removal, relative to fast and super producers.

Nectar reabsorption through perforated/permeable cuticles or stomata on the epidermis (Fahn, 1988; Nepi et al., 2001) has long been known (Bonnier 1878, as cited by Nepi et al., 2001). Resorption of residual nectar recovers otherwise wasted energy (Corbet and Delfosse, 1984; Fahn, 1988; Burquez and Corbet, 1991) and may also protect plants from post-pollination visits by nectar thieves and pollinators that could damage the fertilized flower or plant (Burquez and Corbet, 1991). Nectar resorption is rare in species that accumulate nectar at a site remote from the nectary (Burquez and Corbet, 1991), such as in a nectar spur, because the accumulated nectar does not contact the perforated epidermis of the nectary (however, see Stpiczyńska, 2003c).

Cessation of nectar production immediately after ovule fertilization (cessation of female function) is beneficial if additional visitations will not enhance plant fitness. Many studies support this expectation, showing significant reduction or inhibition of nectar secretion after fertilization (Aizen and Raffaele, 1996; Ladio and Aizen, 1999; Stpiczyńska, 2003b, although see Harder and Barrett, 1992). Aizen and Basilio (1998) reported that pollen removal (cessation of male function) has no effect on nectar inhibition and suggested that low nectar costs and evolutionary constraints may be responsible.



## 1.3 Environmental Influences on Nectar

A plant's environment can strongly affect its nectar characteristics (McCall and Primack, 1992; Corbet et al., 1993). Environmental conditions can change nectar directly after it has been produced. For example, nectar concentration varies negatively with humidity, because of evaporation (Kingsolver and Daniel, 1983), so that nectar tends to be more concentrated in dry environments (Corbet and Delfosse, 1984; Wyatt et al., 1992). Flower shape influences the susceptibility of nectar concentration to changes in atmospheric humidity, so that species with shallow, open flowers, which expose their nectar to evaporation, typically have more viscous nectar than species with deep, tubular flowers (Corbet et al., 1979a; Manetas and Petropoulou, 2000). Corbet et al. (1979a) also found that hairs within flowers can alter the micro-climate and moderate evaporative effects associated with high temperature and wind.

Environmental influences can also affect nectar production indirectly by modifying a plant's ability to produce nectar. Like all enzymatic processes, temperature affects nectar secretion, with peak secretion at an intermediate temperature and reduced secretion at extreme high or low temperatures, because of decreased respiration (Jakobsen and Kristjánsson, 1994; Petanidou and Smets, 1996). Optimal secretion temperatures differ between species (Petanidou and Smets, 1996). Light may also play a role in nectar secretion and quantity, because increased photosynthesis can produce more sugars for nectar secretion, although experiments addressing such an affect could not distinguish light effects from temperature effects (Corbet et al., 1993; Petanidou and Smets, 1996). Studies of nectar production during drought have found dramatic reductions in nectar volume (Carroll et al., 2001), whereas a relative humidity of 100% caused significant increases in volume (Wyatt et al., 1992), indicating that plant

resource availability affects nectar production. Mineral and nutrient enrichment also increase nectar secretion (Petanidou et al., 1999).

Defoliation strongly decreases nectar production. Leaf removal, either experimentally or as a result of herbivory, reduces the carbohydrates produced through photosynthesis that are ultimately used in nectar production (Zimmerman and Pyke, 1988b; Michaud, 1989; Aizen and Raffaele, 1996). Aizen and Raffaele (1996) reported that defoliation of plants reduced nectar production slightly; however, they found no differences between plants with half or all of their leaves removed. Aizen and Raffaele (1996) suggested that carbohydrates stored in the nectaries prior to defoliation may explain this result.

Environmental influences are rarely stable for extended periods. Several studies have examined patterns in nectar volume and concentration during the lifetime of a flower, typically finding rapid nectar secretion when a flower opens, which decreases during the flower's lifetime (Corbet et al., 1979b; Corbet and Delfosse, 1984; Wyatt et al., 1992). In contrast Castellanos et al. (2002) found a constant capacity for nectar secretion during the lives of flowers of two *Penstemon* species. Open flowers of these species secrete nectar until they are full, after which secretion slows to a rate sufficient to replace nectar loss through evaporation. If nectar is removed, secretion resumes at the maximal rate.

## 1.4 Pollinator Responses to Nectar

Nectar-feeding pollinators seek nectar rewards that maximize their rate of net energy intake (Charnov, 1976; Pyke, 1978b,a). As a result, variation in nectar volume among and within plants affects a pollinator's foraging behaviour (Real, 1981; Hodges, 1985; Biernaskie et al., 2002). When moving among plants, pollinators employ

“area-restricted search patterns”, moving short distances and turning often in highly rewarding patches, but moving further and turning less often in poorly rewarding patches (Heinrich, 1979; Morse, 1980; Keasar et al., 1996). Area-restricted searching helps pollinators remain in high-rewarding patches and avoid low-rewarding ones.

Studies of risk-adverse foraging in hummingbirds and bumble bees reveal that pollinators prefer patches with constant rewards over variable patches (Biernaskie et al., 2002). Hodges (1985) suggested that pollinators (particularly bumble bees) use a threshold volume as the basis for deciding when to leave inflorescences. According to this hypothesis, a bee should leave the plant in search of better rewards after probing a flower with relatively little nectar, whereas it should continue foraging on a plant with nectar volumes above the threshold. However, other observations indicate that pollinators are somewhat more tolerant of occasional poorly rewarding flower than implied by a threshold-departure rule, and that their departure behaviour depends on plant architecture. Bees and hawk moths begin foraging at the bottom of vertical inflorescences and progress upwards (Waddington and Heinrich, 1979; Dreisig, 1985). Upward foraging on vertical inflorescences is efficient for pollinators, because energy rewards often follow a gradient within inflorescences (Pyke, 1979). Pollinators can also judge a plant’s reward status from the nectar they extract from the first few flowers, so lower flowers provide information about the upper flowers. For example, nectar absent on low flowers may indicate a poor-quality plant or recent visitation by another pollinator (Waddington, 1981). Upward foraging also ensures few revisits during a foraging bout (Galen and Plowright, 1985b). In contrast, bees visiting heads or umbels remain on these inflorescences until they encounter several non-rewarding flowers (Cresswell, 1990). Harder et al. (2001), suggested that these inflorescence types allow less correlation in reward availability among flowers, requiring pollinators to encounter more flowers to determine a plant’s reward status.

## 1.5 Nectar Secretion as a Strategy

Plants produce floral nectar to manipulate the behaviour of their pollinators, thereby promoting pollen dispersal. In particular, plants that consistently produce more nectar than the population average receive more visits of longer duration and have more flowers probed per pollinator (Zimmerman and Pyke, 1988b; Cresswell, 1999). As a result, these attractive displays receive more pollen from more donors and have more pollen removed by pollinators (Stanton and Preston, 1986). However, these responses can have negative effects on pollen export, so that selection that enhances the quantity and quality of pollination should optimize the amount of nectar available to pollinators to maximize fitness by reducing costs and enhancing pollen removal and deposition (Bell, 1985; Harder and Thomson, 1989).

One cost of increased nectar production arises because greater pollen removal by individual pollinators may not enhance total pollen export by all pollinators (Harder and Thomson, 1989; Harder and Wilson, 1994) as a result of diminishing returns. Pollinators can remove over 80% of a flower's total pollen during a single visit (Strickler, 1979); however, only about 1% of removed pollen reaches conspecific stigmas (Levin and Berube, 1972; Harder and Thomson, 1989; Harder, 2000). This proportion declines as individual pollinators remove more pollen (Harder and Thomson, 1989). Hence, plants that restrict pollen removal by individual pollinators and use many pollinators should export more pollen than those that allow a few pollinators remove most of a flower's pollen (Harder and Thomson, 1989).

In addition, stimulation of visits to many flowers within a plant by individual pollinators by abundant nectar production increases between-flower self-pollination (Barrett et al., 1994; Mitchell et al., 2004). The pollen used in geitonogamy typically reduces pollen that otherwise could have been exported to other plants

(pollen discounting: Harder and Barrett, 1995). In addition, self-pollination increases the incidence of inbreeding depression for the progeny of self-compatible plants (Charlesworth et al., 1987; Charlesworth and Charlesworth, 1987b).

## 1.6 Empty Flowers

Production of nectarless (empty) flowers may effectively enhance pollination, while limiting energy costs. Nectarless flowers occur in three patterns. First, a species can produce no nectar in any flowers, as is the case for approximately one-third (8000 species) of orchid species (Dressler, 1981). Second, within a species some individuals produce nectar, whereas others do not. For example, Golubov et al. (1999) reported that 54% of plants in *Prosopis glandulosa* populations produced no nectar. Third, some flowers within a plant may remain empty. Feinsinger (1978) first reported this phenomenon in five tropical species of hummingbird-pollinated plants, with 3 to 61% of a plant's flowers producing no nectar. Such empty flowers in nectar-producing species may enhance reproduction (Bell, 1986), although this effect has not been tested.

## 1.7 Thesis Objectives

In this thesis, I examine the role of nectarless flowers in nectar producing species in an ecological and evolutionary context. The remainder of this thesis comprises four chapters that address empty flowers for complementary perspectives:

- Chapter 2 explores the incidence of empty flowers among plant species and assess whether the pattern of variation is consistent with saving energy and/or direct enhancement of pollination.

- Chapter 3 describes a manipulative experiment that tests the influences of the proportion and location of empty flowers within fireweed (*Chamerion angustifolium*) inflorescences on the behaviour of pollinating bumble bees (*Bombus sp.*).
- Chapter 4 reports on a similar experiment to assess the effects of the proportion of empty flowers within inflorescences of a moth-pollinated orchid (*Platanthera dilatata*) on self-pollination and pollen export.
- Finally, Chapter 5 considers the implications of the findings described in the preceding chapters for the strategic evolution of empty flowers.

Overall, this thesis provides a comprehensive examination of the occurrence of nectarless flowers and their influences on pollinator behaviour and pollen transfer. Accordingly, this research makes important contributions to our understanding of the evolutionary ecology of pollinator behaviour and plant reproduction.

# Chapter 2

## The Incidence of Empty Flowers

### 2.1 The Purpose of Empty Flowers

Sexual reproduction is difficult for plants, because of their immobility, so that plants require vectors to import and export pollen for reproduction. Most angiosperm species (80%) involve animal pollinators in this process (Ackerman, 2000), which typically entails the production of a reward to encourage pollinators to visit flowers of the same species consistently, thereby facilitating pollen transfer. To serve this purpose, animal-pollinated angiosperms typically offer floral nectar (Simpson and Neff, 1983; Ackerman et al., 1994; Stpiczyńska, 2003a), which is produced by nectaries typically located within flowers to ensure that pollinators contact anthers and stigmas for pollen exchange.

In addition to its role in pollinator attraction, nectar provides a mechanism by which plants can manipulate the behaviour of attracted pollinators. The number of flowers probed by a pollinator and probe duration increase with nectar volume (Zimmerman and Pyke, 1988b; Cresswell, 1999), leading to higher pollen removal and deposition (Thomson and Plowright, 1980; Hodges and Wolf, 1981; Harder and

Thomson, 1989).

A variety of mating and energetic costs counteract the benefits of nectar production. Increased visit duration can reduce reproductive success by increasing within-plant self-pollination (geitonogamy: Harder et al., 2001) and pollen loss during transport (Harder and Thomson, 1989). Furthermore, the benefits of nectar production are somewhat offset by the associated costs of production, which differ considerably among species. Southwick (1984) reported that energy investment in nectar by *Asclepias syriaca* represented 37% of a plant's total photosynthetic energy during flowering. Consequently, high investment in nectar production can reduce seed production and plant display size (Zimmerman and Pyke, 1988a; Teuber et al., 1990; Pyke, 1991). In contrast, nectar production accounts for only 3% of the total energy cost of *Pontederia cordata* flowers (Harder and Barrett, 1992) and in some species the costs of nectar production is completely offset by photosynthesis by the flower itself (Zimmerman, 1988a).

Natural selection should balance the benefits and costs of nectar production to maximize plant fitness (Harder, 1988; Keasar et al., 1996), in some cases leading to no nectar production. Three patterns in the occurrence of nectarless flowers are found in angiosperm populations. First, in some species no plants produce nectar. Such nectarless species either rely on other floral products, such as pollen (Buchmann, 1983) or floral oils (Simpson and Neff, 1981), to reward pollinators, or they deceive pollinators into visiting based on their learned associations with other rewarding plants, or mates (Johnson et al., 2003a,b). Deceit pollination is rare among angiosperms with granular pollen, but characterizes approximately a third of all orchids (8000 species: Dressler, 1981). Second, some plants within a species may be nectarless, whereas others are not. For example, nectar-producing individuals of *Prosopis glandulosa* attract more pollinators, whereas nectarless trees produce 7%



more pollen, so that production of nectar or not seem to be alternate reproductive strategies in this species (Golubov et al., 1999). The third pattern involves individuals that produce a proportion of nectarless, or empty flowers within their inflorescences (Feinsinger, 1978).

## 2.2 Empty Flowers as a Strategy

Two hypotheses have been proposed to explain the production of some empty flowers by individual plants. Bell (1986) characterized empty flowers as an energy-saving strategy. This hypothesis predicts that empty flowers should be most common among species with high energy costs of nectar production, and that the proportion of empty flowers should vary positively with floral costs. In general, nectar production varies negatively with inflorescence size and positively with flower size (Harder and Cruzan, 1990; Harder and Barrett, 1992). Therefore, large-flowered species with large displays should incur higher energy demands and should benefit more from producing some empty flowers than do species with lower investments (Figure 2.1). Plants with empty flowers should still benefit from pollen transfer either if pollinators cannot discriminate between rewarding and non-rewarding flowers, or if the energy that pollinators expend in discrimination exceeds the cost of probing an adjacent flower.

Unlike the energy-saving hypothesis, the pollinator-manipulation hypothesis proposes that empty flowers enhance pollination directly. Large floral displays promote pollinator attraction (Ohashi and Yahara, 2001) but can increase geitonogamy (Harder and Barrett, 1995), which reduces opportunities for pollen export (pollen discounting: Lloyd, 1992; Harder and Barrett, 1995) and increases the incidence of inbreeding depression for self-compatible species (Charlesworth and Charlesworth, 1987a). Pollinators decide to leave inflorescences based on the amount

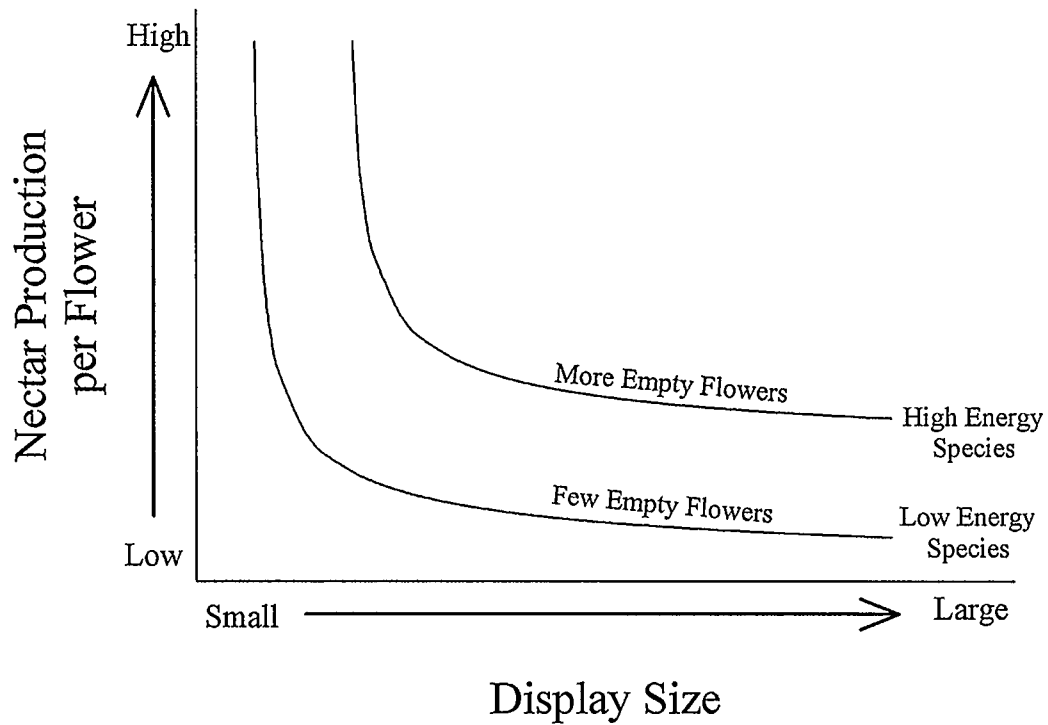


Figure 2.1: Empty flowers as an energy-saving strategy. Species that invest extensively in nectar production should produce more empty flowers than those with low nectar costs to save energy.

of nectar encountered in flowers and the number of visits varies negatively with nectar production (Hodges, 1985; Dreisig, 1989; Cresswell, 1990). By limiting geitonogamy and its associated costs, the use of empty flowers to manipulate pollinator behaviour should be most beneficial for species that display many flowers (Figure 2.2).

The proportion of empty flowers that optimally manipulates pollinator behavior should depend on pollinator abundance. The proportion of pollen reaching stigmas varies negatively with the amount of pollen removed, so that a plant could maximize pollen dispersal by limiting pollen removal by individual pollinators and allowing several pollinators to remove available pollen (Harder and Thomson, 1989; Harder

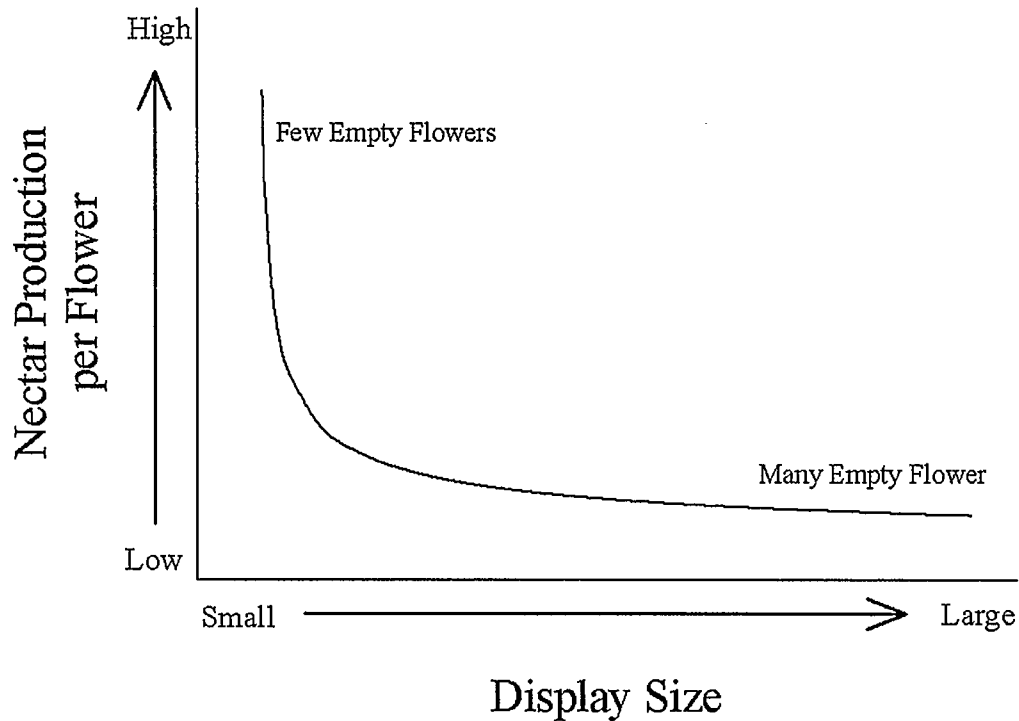


Figure 2.2: Empty flowers as a pollination-enhancement strategy. Species that display many flowers are more susceptible to self pollination and so benefit more by using empty flowers to encourage a pollinator to leave in search of better rewards

and Wilson, 1994). With few pollinators, pollen removal should not be restricted, so that empty flowers will not be advantageous. If pollinators are abundant, restricted pollen removal by individual pollinators promotes total pollen export, so some empty flowers would be beneficial (Harder and Thomson, 1989; Harder and Wilson, 1994). However, some nectar-producing flowers are needed because pollinators encountering inflorescences with all flowers empty would learn avoidance, resulting in low visitation and reproductive success (Smithson and Gigord, 2003).

The incidence of empty flowers in nectar-producing species is poorly known, so the energy-saving and pollinator-manipulation hypotheses remain to be tested. Feinsinger

(1978) reported that between 3 and 61% of flowers of five tropical species pollinated by hummingbirds produced no nectar. Biernaskie and Cartar (2004) examined variation in nectar production by nine legume species in Alberta and reported a positive relation with floral display size, although they did not report explicitly on empty flowers. Given this lack of information, I surveyed nectar production by 52 plant species to assess the incidence of empty flowers and whether it varies as predicted by the energy-saving and/or pollinator-manipulation hypotheses.

## 2.3 Materials and Methods

### 2.3.1 Study Design

I sampled nectar production in 52 species from western Alberta and British Columbia during the summers of 2004 and 2005. Randomly selected plants within a population were bagged with nylon-mesh bags to exclude pollinators, allowing nectar to replenish in nectar-producing flowers. Bags were placed on enough plants to include 100 flowers on at least 10 plants; otherwise all flowers were bagged. For example, in a population in which plants displayed 10 open flowers, 10 plants would be bagged (10 flowers/plant x 10 plants = 100 bagged flowers).

### Estimation of Energy Investment

The total energy that a species invests in flowering was estimated by summing the energy costs of flower construction, flower maintenance and nectar production. Floral construction costs were estimated by weighing the dry mass of 10 flowers per species. Flowers were dried for two days at 35°C in a drying oven to avoid the loss of volatile compounds that would occur at higher temperatures (Petanidou and Vokou, 1990).

I converted the average flower mass to construction energy by multiplying by the average mass-specific energy content of flowers reported in the literature. Published studies of floral energy content in 27 species reveal little variation, with a mean ( $\pm$ SD) of 20.27 ( $\pm$ 1.38) KJ/gram (Table 2.1). To determine a species' total flower construction costs I multiplied the average cost per flower by the average number of open flowers.

Energy costs of flower maintenance were estimated for 34 of the 52 species as a linear function of floral longevity. I quantified floral longevity for one flower on each of 20 plants per species. A flower bud was marked with adhesive tape on the stem or petiole (depending on species) as it was about to open. I then monitored the flower daily until it ceased being effective for pollination, either because it fell off the plant with light tapping or it had wilted so that either a pollinator could not probe for nectar, or the anthers were wilted and showed no pollen and/or the stigma had turned brown. A bud that failed to open was abandoned and replaced with a new bud on a different plant. Maintenance cost per flower was calculated by multiplying a species' average floral longevity, its average flower mass and the average daily, mass-specific carbon loss for flower maintenance of 0.0133 mg of carbon per day per gram (reported by Ashman and Schoen, 1994 for 10 species).

To assess nectar costs, I measured 24-h sugar production per flower in one of two ways. For species that produce little nectar per flower ( $<0.5 \mu\text{L}$ ), I used the Anthrone technique (McKenna and Thomson, 1988) to quantify the sugar in nectar absorbed on filter paper wicks. After extracting nectar from a flower, I dried the wicks and mounted them on insect pins in a box until they could be analyzed. In the laboratory, I re-dissolved the nectar in boiling distilled water and added anthrone reagent to induce a colour change, which was then measured spectrophotometrically and compared against samples with known nectar contents (see McKenna and Thomson, 1988, for

Table 2.1: A summary of published reports of the energy content of dry flowers (KJ/gram) estimated by bomb calorimetry for 27 species.

Species	Energy(KJ/gram)	Source
<i>Pontederia cordata</i>	19.06	Harder and Barrett (1992)
<i>Espeletia schultzei</i>	21.01	Baruch (1982)
<i>Coespeletia lutescens</i>	21.16	Baruch (1982)
<i>Coespeletia moritziana</i>	20.98	Baruch (1982)
<i>Coespeletia spicata</i>	21.88	Baruch (1982)
<i>Hypericum laricifolium</i>	21.54	Baruch (1982)
<i>Castilleja fassifolia</i>	21.91	Baruch (1982)
<i>Senecio formosus</i>	20.14	Baruch (1982)
<i>Hinterhuberia imbricata</i>	21.69	Baruch (1982)
<i>Draba lindenii</i>	20.08	Baruch (1982)
<i>Lupinus meridanus</i>	20.53	Baruch (1982)
<i>Orthosanthus chimboracensis</i>	20.67	Baruch (1982)
<i>Trifolium parryi</i>	15.53	Smith (1969)
<i>Argoseris glauca</i>	18.21	Smith (1969)
<i>Arabis drummondii</i>	20.64	Andersen and Armitage (1976)
<i>Draba aurea</i>	20.55	Andersen and Armitage (1976)
<i>Erysimum asperum</i>	20.52	Andersen and Armitage (1976)
<i>Thlaspi alpestre</i>	20.11	Andersen and Armitage (1976)
<i>Erythronium grandiflorum</i>	20.11	Andersen and Armitage (1976)
<i>Zygadenus elegans</i>	20.16	Andersen and Armitage (1976)
<i>Lewisia pygmaea</i>	17.39	Andersen and Armitage (1976)
<i>Caltha leptosepala</i>	20.59	Andersen and Armitage (1976)
<i>Ranunculus sp.</i>	19.99	Andersen and Armitage (1976)
<i>Sibbaldia procumbens</i>	20.58	Andersen and Armitage (1976)
<i>Penstemon whippleanus</i>	20.18	Andersen and Armitage (1976)
<i>Veronica wormskholdii</i>	21.20	Andersen and Armitage (1976)
<i>Pseudocymopterus montanus</i>	20.75	Andersen and Armitage (1976)

complete details).

For species that produce larger nectar volumes per flower ( $>0.5 \mu\text{L}$ ) I measured nectar volume and concentration directly. Nectar was extracted with calibrated micro-capillary tubes and the volume determined from the length of the nectar column within the tube (measured using digital calipers) relative to the tube's length. Nectar concentration (mg solute/mg solution) was measured in the field using a Bellingham and Stanley sugar refractometer that had been adjusted to accommodate small volumes. Total energy content of the nectar was calculated as the product of nectar volume, concentration, density and the energy content of sucrose (16.48 joules/mg: Kearns and Inouye, 1993). Total sugar production during a flower's life was estimated from the average sugar production per flower and average floral longevity.

### Plant and Population Characteristics

In addition to determining each species' energy investment in flowering and their proportion of empty flowers, I measured several other plant characteristics to assess the energy-saving and pollination-enhancement hypotheses. I counted the open flowers per inflorescence (hereafter referred to as display size) and on entire plants. Bell's (1986) energy-savings hypothesis proposes that the incidence of empty flowers increases with flower size (Figure 2.1) because of pollinator discrimination difficulties, so I measured corolla lengths with digital calipers. Inflorescence architecture may affect the delivery of resources to individual flowers (Wyatt, 1982), so I classified species according to four inflorescence architectures: branched inflorescences (i.e. panicles), vertical unbranched inflorescences (i.e. racemes), solitary flowers, and umbels and capitula (Figure 2.3). Pollinator observations were also conducted to determine the primary pollinator types for each species. I also measured population size and area to determine the population density, and location, elevation and

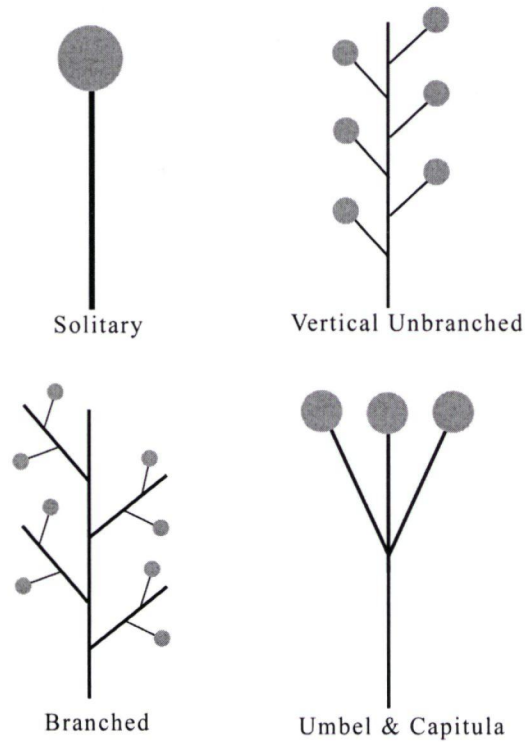


Figure 2.3: General examples of the four classes of inflorescence architectures (solitary, vertical unbranched, branched and umbels and capitula). Each circle depicts a flower

qualitative assessments of site characteristics that might affect growth conditions. To quantify the average nectar reward available to pollinators in a population I measured nectar standing crop. Time of sampling and temperature were also recorded to determine whether these environmental factors affect nectar production and characteristics.

### 2.3.2 Analysis

I used general linear models (Kutner et al., 2005) of species means for all analyses. These analyses typically considered ln-transformed variables to normalize the



residuals and enhance the linearity of the relations. Rather than analyzing variation in the proportion of empty flowers directly, I considered the average proportion of nectariferous flowers per inflorescence for each species. I adopted this approach because many inflorescences had no empty flowers, so that ln-transformation of the proportion of empty flowers was not possible. After identifying significant effects of categorical factors, I used Dunn-Šidák multiple comparisons to test for differences between specific categories.

To illustrate the independent effects of variables in analyses that detected multiple effects on a dependent variable, I adjusted individual observations to exclude the influences of all significant independent variables other than a particular predictor of interest. This adjustment was based on the general linear model estimated for a specific dependent variable. For example, consider a situation in which a dependent variable ( $Y_i$ ) for observation  $i$  is influenced by two independent variables ( $X_{1,i}$  and  $X_{2,i}$ ) and has a residual ( $e_i$ ). To examine the relation between the dependent variable and  $X_1$ , the other independent variable is held constant by replacing its value for observation  $i$  with the mean  $\bar{X}_2$ , resulting in an adjusted dependent value of

$$\tilde{Y}_{i|2} = a + b_1 X_1 + b_2 \bar{X}_2 + e_i \quad (2.1)$$

where  $a$  is the intercept, and  $b_1$  and  $b_2$  are estimated partial regression coefficients for independent variables  $X_1$  and  $X_2$ , respectively. Similarly, the influence of  $X_2$  is illustrated by the adjusted dependent values ( $\tilde{Y}_{i|1}$ ) with the mean value of the first independent variable ( $\bar{X}_1$ ).

## 2.4 Results

### 2.4.1 Interspecific Variation in Display Size and Flowering Costs

Fifty-two species, from 23 families and 43 genera were examined for nectar production. The average ( $\pm$ SD) floral display of these species ranged from 1 ( $\pm$ 0) flower in *Lilium philadelphicum* to 45.9 ( $\pm$ 18.78) open flowers in *Trifolium pratense* (Figure 2.4). Total floral energy investment ranged two orders of magnitude between 37.9 joules in *Trifolium pratense* and 4067 joules for *Lilium philadelphicum*, with a mean ( $\pm$ SD) of 464 ( $\pm$ 750) joules (Table 2.2, Figure 2.5a). Energy investment per flower varied negatively with display size ( $F_{1,32}=9.43$ ,  $P<0.005$ ,  $R^2=0.23$ ; Figure 2.6).

Flower production, maintenance and nectar production contributed unequally to the total cost of flowering. Floral construction costs (as measured by flower mass) varied little within species, but extensively among species (Table 2.2). Flower production accounted for the largest component of flowering costs, with a mean ( $\pm$ SD) of 88 ( $\pm$ 2.8)% and ranged from 80% in *Lonicera ciliosa* to 96% in *Elaeagnus commutata* (Figure 2.5b). Floral maintenance was examined in 34 species and demanded the second highest energy investment during flowering, with a mean ( $\pm$ SD) of 8.5 ( $\pm$ 3.3)% and a range from 2% in *Elaeagnus commutata* to 16% in *Delphinium glaucum* ((Table 2.2), Figure 2.5c). Finally, nectar production (as measured by total sugar) required the least energy investment, with a mean ( $\pm$ SD) of 3.3 ( $\pm$ 2.3)% and a range from 0.1% of total floral costs in *Cytisus scoparius* to 8% in *Lonicera dioica* ((Table 2.2, Figure 2.5d). Mean nectar production did not vary significantly with mean display size for the 52 species ( $F_{1,50}=0.13$ ,  $P>0.50$ ,  $R^2=0.003$ ; Figure 2.7). However, species with small flowers invested a larger proportion of total floral energy

Table 2.2: Estimates of the energy per flower and its percentage distribution among production, maintenance and nectar costs for 52 species sampled in southern British Columbia and southwestern Alberta.

Species	Floral Energy (j)	Percent Energy Investment		
		Production	Maintenance	Nectar
<i>Allium cernuum</i>	75.50	84.20	8.96	6.84
<i>Allium schoenoprasum</i>	87.15	88.57	5.20	6.22
<i>Aquilegia flavescens</i>	1036.49	85.80	9.13	5.07
<i>Arabis holboellii</i>	63.57	86.28	11.86	1.86
<i>Arctostaphylos uva-ursi</i>	131.26	86.97	8.84	4.16
<i>Astragalus eucosmus</i>	76.03	90.84	6.40	2.76
<i>Campanula rotundifolia</i>	393.62	91.91	6.26	1.83
<i>Castilleja miniata</i>	563.94	80.61	14.15	5.23
<i>Cerastium arvense</i>	102.69	87.03	11.63	1.32
<i>Chamerion angustifolium</i>	608.51	90.90	6.65	2.45
<i>Chamerion latifolium</i>	1115.96	92.52	6.77	0.71
<i>Corydalis aurea</i>	80.97	87.31	5.46	7.23
<i>Delphinium bicolor</i>	960.63	83.51	15.05	1.45
<i>Delphinium glaucum</i>	676.00	89.58	6.31	4.11
<i>Elaeagnus commutata</i>	121.61	86.85	11.55	1.59
<i>Erythronium grandiflorum</i>	923.37	88.18	11.43	0.38
<i>Geum triflorum</i>	1206.12	83.50	15.99	0.50
<i>Hedysarum alpinum</i>	78.64	94.29	2.51	3.21
<i>Hedysarum boreale</i>	298.37	87.82	10.10	2.07
<i>Hedysarum sulphurescens</i>	154.36	85.41	7.67	6.93
<i>Lathyrus ochroleucus</i>	283.97	87.13	6.37	6.50
<i>Lilium philadelphicum</i>	4066.90	86.27	13.19	0.54
<i>Lonicera dioica</i>	245.17	86.17	6.19	7.64
<i>Medicago sativa</i>	61.75	91.45	6.89	1.66
<i>Mertensia paniculata</i>	191.66	88.23	6.63	5.13
<i>Monarda fistulosa</i>	76.23	89.27	6.77	3.97
<i>Oxytropis sericea</i>	1434.57	87.65	11.83	0.52

Table 2.2: Continued

Species	Floral Energy (j)	Percent Energy Investment		
		Production	Maintenance	Nectar Cost
<i>Pedicularis bracteosa</i>	149.50	90.09	6.50	3.42
<i>Penstemon confertus</i>	62.70	89.41	4.13	6.46
<i>Penstemon nitidus</i>	167.45	90.13	7.85	2.01
<i>Phacelia sericea</i>	94.52	90.48	8.60	0.92
<i>Pinguicula vulgaris</i>	74.84	86.85	12.37	0.78
<i>Polemonium pulcherrimum</i>	77.72	91.47	6.20	2.33
<i>Trifolium pratense</i>	37.92	88.40	6.58	5.02

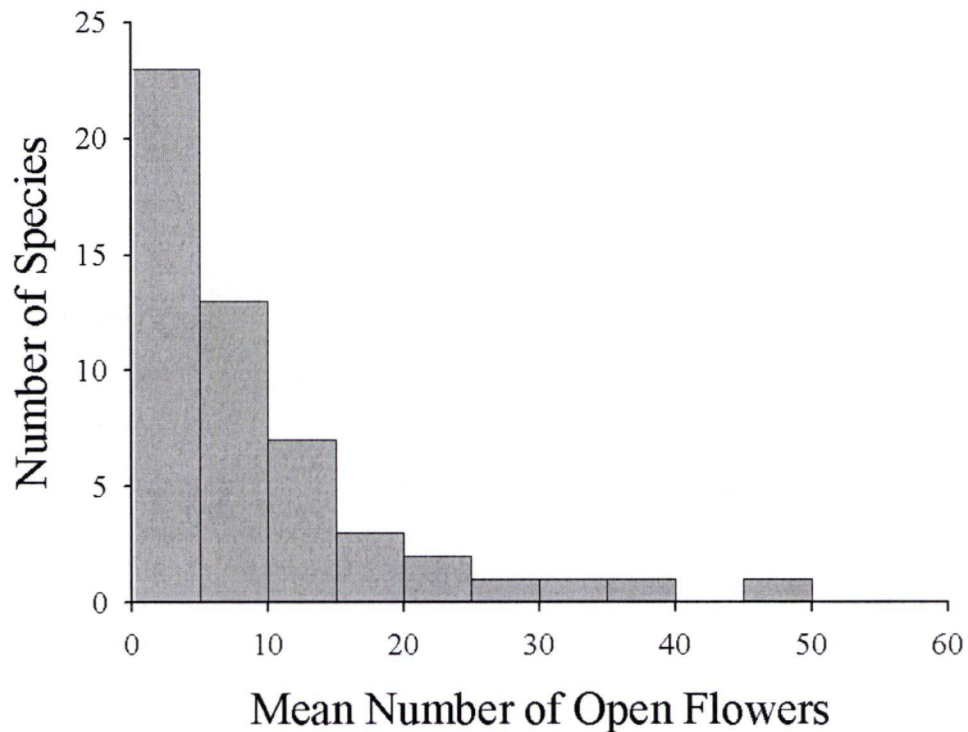


Figure 2.4: Variation in mean display size for 52 species from western Alberta and British Columbia.

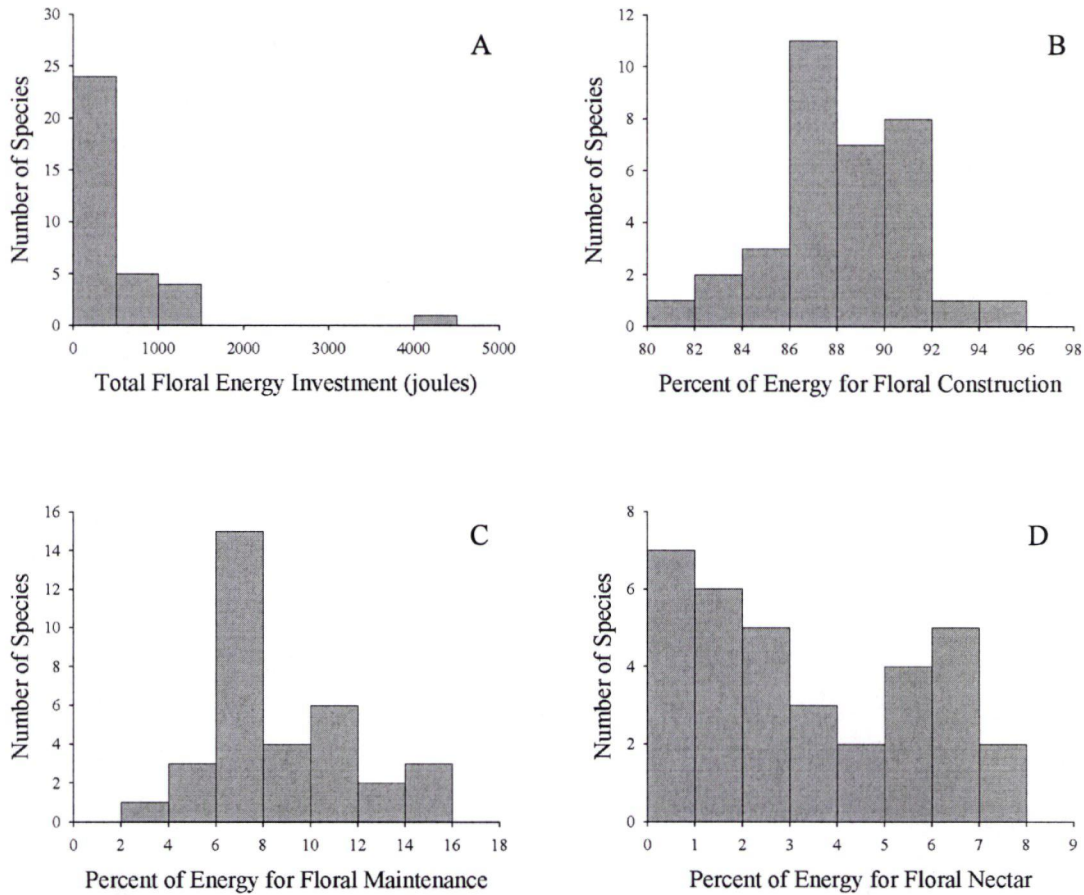


Figure 2.5: Variation in the costs of flowering for 52 species, including a) total energy investment per flower, and the percentages of investment in b) floral production, c) floral maintenance and d) nectar production.

in nectar production than did large-flowered species ( $F_{1,32}=9.50$ ,  $P<0.005$ ,  $R^2=0.23$ : Figure 2.8).

### 2.4.2 Influences of the Incidence of Empty Flowers

Of the 52 sampled species, 42 (81%) contained nectarless flowers, with the average species producing 11 ( $\pm 2$ )% empty flowers (Table ??, Figure 2.9). Among species with nectarless flowers, *Zigadenus venenosus* had the lowest proportion of empty flowers

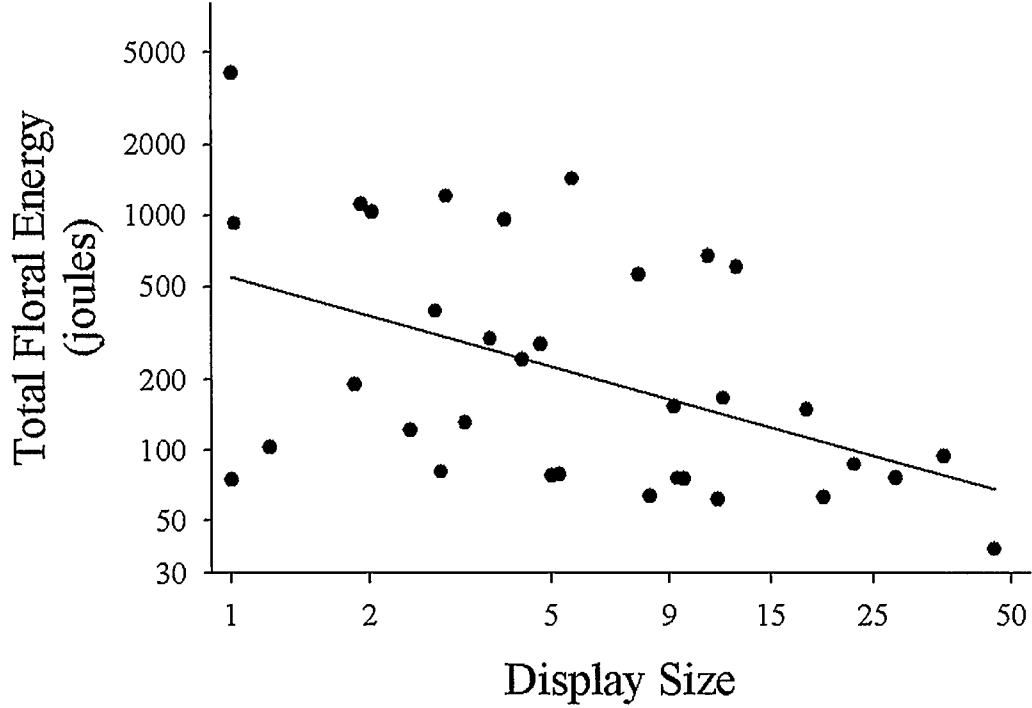


Figure 2.6: The relation of average energy investment per flower (joules) to mean display size for 52 species. Note ln-scaled axes.

with 1% and *Linnaea borealis* had the largest proportion of 56%. The proportion of empty flowers did not differ among taxonomic families ( $F_{26,25}=0.63$ ,  $P>0.75$ ); however it did vary significantly among genera within families ( $F_{42,9}=3.15$ ,  $P<0.05$ ,  $R^2=0.94$ ). The latter conclusion should be interpreted with caution, as it depends on inclusion of *Linnaea borealis* in the analysis ( $F_{41,9}=2.14$ ,  $P>0.10$ ).

The mean proportion of nectariferous flowers varied significantly among species with several aspects of flower production (Table 2.4). Both average sugar production per flower and plant architecture affected the proportion of nectariferous flowers strongly, whereas average inflorescence display size had a weaker influence ( $F_{15,35}=3.84$ ,  $P=0.0005$ ,  $R^2=0.62$ ). However, most influences on the production of

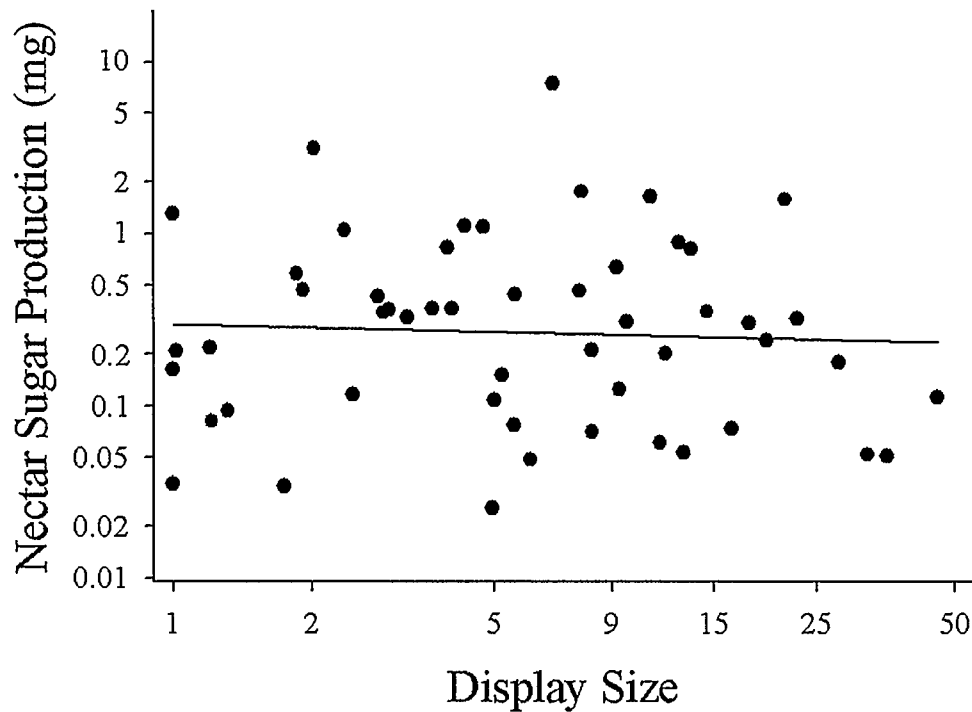


Figure 2.7: The relation of nectar production to mean display size of 52 species. Note ln-scaled axes.

nectariferous flowers were more complex, as indicated by significant interactions of total sugar production per flower with display size and flower mass. In particular, flower size affected the proportion of empty flowers positively for species that produce little nectar, but negatively for species that produce considerable nectar (Figure 2.10a). In contrast, display size affected the proportion of empty flowers negatively for species that produce little nectar, but positively for those that produce considerable nectar (Figure 2.10b). Note that the proportion of nectariferous flowers did not vary significantly with the costs of floral maintenance (Table 2.4).

Inflorescence architecture also affected the proportion of empty flowers in a complex manner. Overall, species with umbels or capitula had the highest proportion of empty flowers with a mean ( $\pm$ SE) of 15 ( $\pm$ 1.51)%, compared to species with

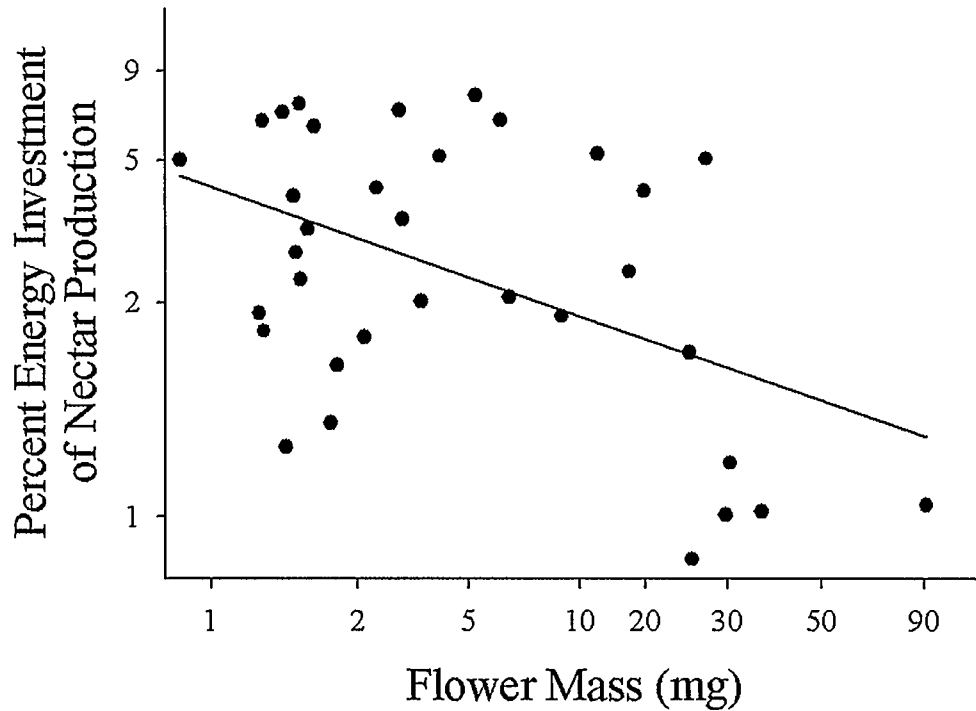


Figure 2.8: The relation of the proportion of energy invested in nectar production to flower size.

unbranched, vertical inflorescence architectures ( $12 \pm 1.15\%$ ), those with solitary flowers ( $5 \pm 1.30\%$ ) and branched inflorescences ( $1 \pm 1.16\%$ ). However, the effects of inflorescence architecture on the proportion of empty flowers depended on a species' sugar production per flower and the elevation of the site at which a species was sampled (Table 2.4). After accounting for the other interactions involving sugar production, the proportion of empty flowers varied negatively among species with sugar production per flower for species with single flowers, vertical, unbranched inflorescences, umbels or capitula (Figure 2.11). In contrast, for species with branched inflorescences, such as panicles and cymes, the proportion of empty flowers varied positively with mean sugar production per flower (Figure 2.11). Curiously,



Table 2.3: The proportion of empty flowers and average display size for 52 species sampled throughout southern British Columbia and southwestern Alberta.

Species	Family	Percent Empty Flowers	Display Size
<i>Allium cernuum</i>	Alliaceae	0.00	9.69
<i>Allium schoenoprasum</i>	Alliaceae	12.44	22.75
<i>Aquilegia brevistyla</i>	Ranunculaceae	29.52	1.20
<i>Aquilegia flavescens</i>	Ranunculaceae	1.06	2.03
<i>Arabis holboellii</i>	Brassicaceae	13.61	8.17
<i>Arctostaphylos uva-ursi</i>	Ericaceae	0.00	3.24
<i>Astragalus eucosmus</i>	Fabaceae	20.39	9.36
<i>Camassia quamash</i>	Agavaceae	13.65	4.05
<i>Campanula rotundifolia</i>	Campanulaceae	9.51	2.79
<i>Castilleja miniata</i>	Orobanchaceae	6.07	7.75
<i>Cerastium arvense</i>	Caryophyllaceae	0.00	1.21
<i>Chamerion angustifolium</i>	Onagraceae	0.00	12.64
<i>Chamerion latifolium</i>	Onagraceae	10.34	1.92
<i>Corydalis aurea</i>	Fumariaceae	3.50	2.86
<i>Cytisus scoparius</i>	Fabaceae	39.61	6.00
<i>Delphinium bicolor</i>	Ranunculaceae	16.80	3.95
<i>Delphinium glaucum</i>	Ranunculaceae	1.11	10.96
<i>Elaeagnus commutata</i>	Elaeagnaceae	23.73	2.46
<i>Erythronium grandiflorum</i>	Liliaceae	37.12	1.02
<i>Geranium viscosissimum</i>	Geraniaceae	17.13	2.36
<i>Geum triflorum</i>	Rosaceae	2.08	2.94
<i>Hedysarum alpinum</i>	Fabaceae	2.24	5.20
<i>Hedysarum boreale</i>	Fabaceae	3.44	3.67
<i>Hedysarum sulphurescens</i>	Fabaceae	13.64	9.24
<i>Heuchera cylindrica</i>	Saxifragaceae	41.67	14.50
<i>Ipomopsis aggregata</i>	Polemoniaceae	0.00	21.40
<i>Lathyrus ochroleucus</i>	Fabaceae	5.45	4.73
<i>Ledum groenlandicum</i>	Ericaceae	7.00	12.90
<i>Lilium philadelphicum</i>	Liliaceae	0.00	1.00

Table 2.3: Continued

Species	Family	Percent Empty Flowers	Display Size
<i>Linnaea borealis</i>	Caprifoliaceae	55.93	1.75
<i>Lonicera ciliosa</i>	Caprifoliaceae	1.79	6.71
<i>Lonicera dioica</i>	Caprifoliaceae	5.36	4.32
<i>Maianthemum stellatum</i>	Ruscaceae	5.09	4.94
<i>Medicago sativa</i>	Fabaceae	10.25	11.46
<i>Mertensia paniculata</i>	Boraginaceae	3.26	1.86
<i>Monarda fistulosa</i>	Lamiaceae	4.00	28.00
<i>Oxytropis sericea</i>	Fabaceae	16.99	5.55
<i>Pedicularis bracteosa</i>	Orobanchaceae	4.29	17.92
<i>Penstemon confertus</i>	Plantaginaceae	0.00	19.50
<i>Penstemon nitidus</i>	Plantaginaceae	5.86	11.78
<i>Phacelia sericea</i>	Boraginaceae	0.00	35.63
<i>Pinguicula vulgaris</i>	Lentibulariaceae	6.19	1.00
<i>Platanthera dilatata</i>	Orchidaceae	10.00	32.30
<i>Polemonium pulcherrimum</i>	Polemoniaceae	3.90	5.00
<i>Prunella vulgaris</i>	Lamiaceae	0.00	8.15
<i>Pulsatilla patens</i>	Ranunculaceae	41.03	1.00
<i>Rhinanthus minor</i>	Orobanchaceae	11.76	5.53
<i>Ribes cereum</i>	Grossulariaceae	30.38	13.38
<i>Trifolium pratense</i>	Fabaceae	0.00	45.88
<i>Viola rugulosa</i>	Violaceae	1.32	1.32
<i>Zigadenus elegans</i>	Melanthiaceae	1.67	7.67
<i>Zigadenus venenosus</i>	Melanthiaceae	1.00	16.40

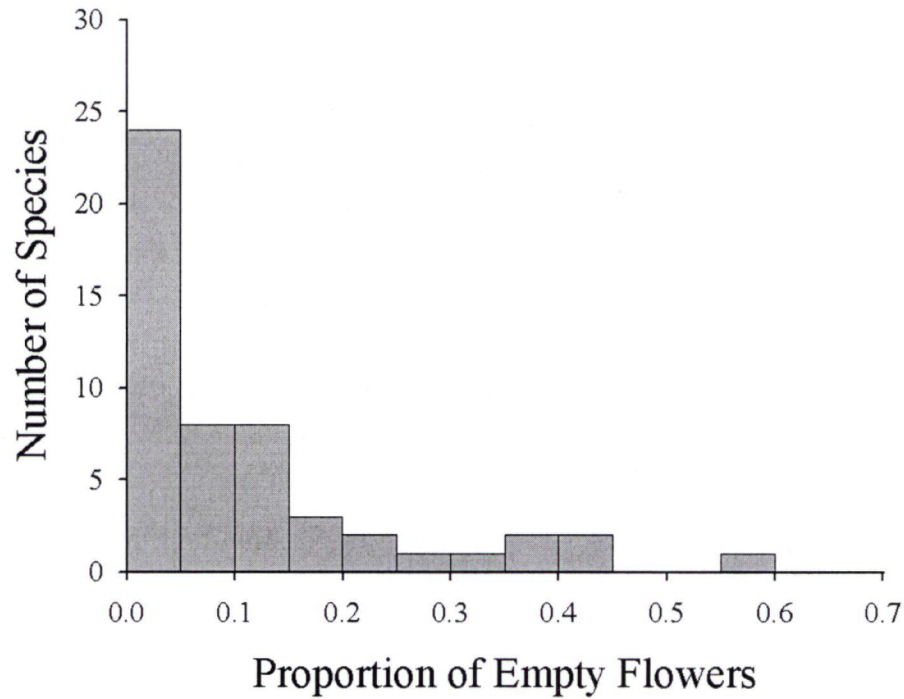


Figure 2.9: Variation in the proportion of empty flowers for 52 species.

Table 2.4: Influences on interspecific variation in the proportion of nectariferous flowers, as indicated by partial  $F$ -tests for a general linear model and partial regression coefficients ( $\pm$ SE) (35 error degrees of freedom).

Variable	$F$	$df$	$P$	Partial Regression	SE
Floral Mass	0.07	1	>0.75	-0.0104	0.0399
Nectar Energy	14.94	1	0.0005	0.4285	0.1108
Display Size	3.90	1	0.05	-0.1123	0.0569
Architecture	4.04	3	<0.05	N/A	N/A
Elevation	1.31	1	>0.25	-0.1465	0.1278
Total Sugar*Display Size	9.88	1	<0.005	-0.0877	0.0279
Total Sugar*Flower Mass	6.13	1	<0.05	0.0513	0.0207
Total Sugar*Architecture	6.47	3	<0.005	N/A	N/A
Elevation*Architecture	4.25	3	<0.05	N/A	N/A

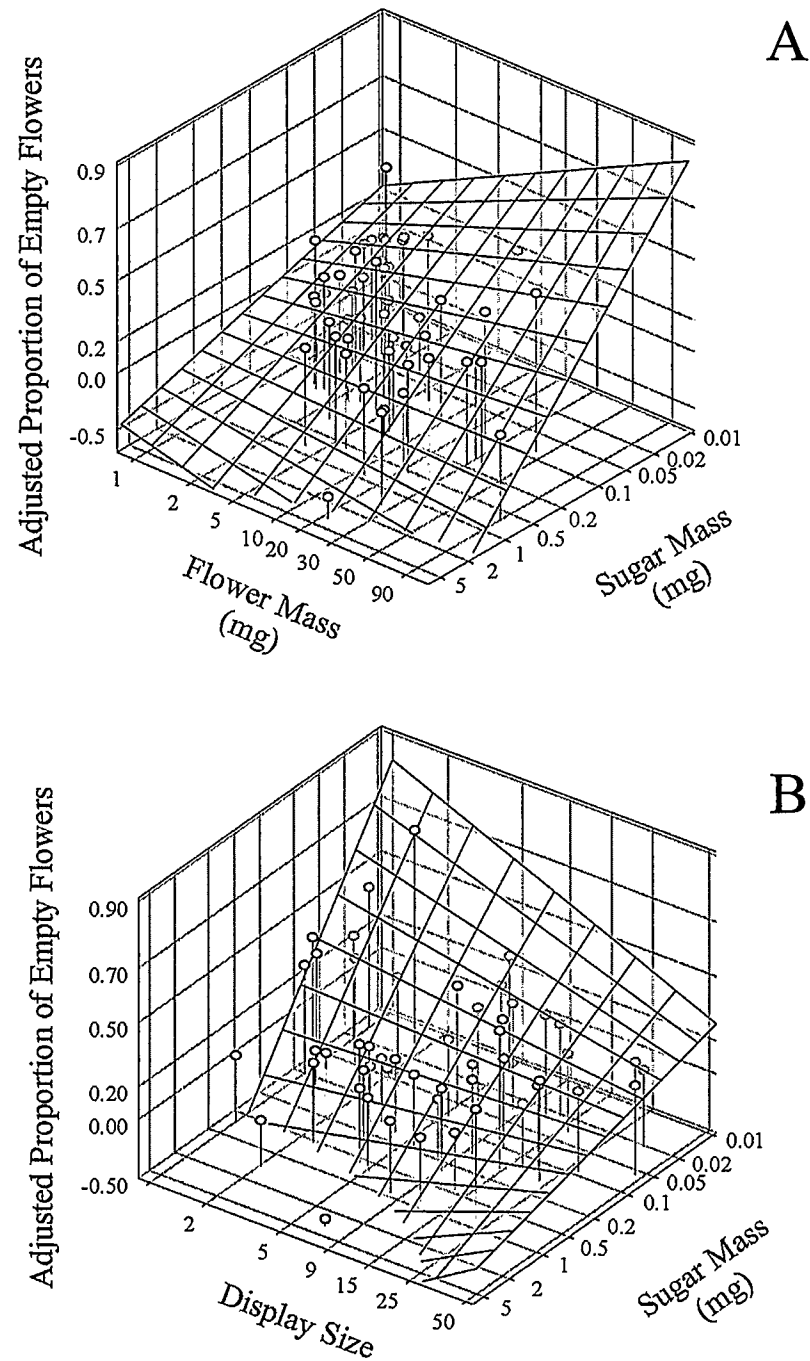


Figure 2.10: Relations of the adjusted proportion of empty flowers for 52 species to the interacting effects of a) flower mass and sugar production per flower, and b) display size and sugar production per flower. For statistical details see Table 2.4.

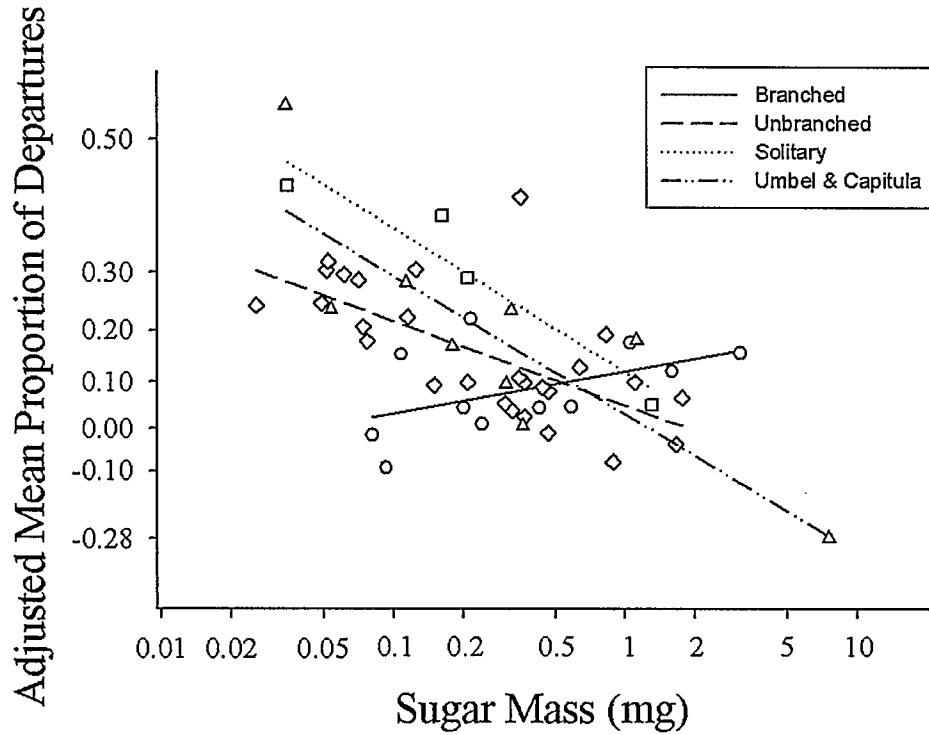


Figure 2.11: The relations of the adjusted proportion of empty flowers to mean sugar production per flower for 52 plant species with different inflorescence architectures, including branched inflorescences (e.g. panicles and cymes:  $\circ$ ), vertical unbranched inflorescences (i.e. spikes and racemes:  $\diamond$ ), solitary flowers ( $\square$ ) and umbels and capitula ( $\triangle$ ).

inflorescence architecture also influenced the relation of the proportion of nectariferous flowers to elevation (Table 2.4). In particular, the proportion of nectariferous flowers increased with elevation for species with umbels and capitula (partial regression coefficient  $b \pm \text{SE} = 0.163 \pm 0.065$ ,  $t_{35}=2.50$ ,  $P<0.05$ ), but decreased with elevation for species with branched inflorescences ( $b \pm \text{SE} = -0.261 \pm 0.115$ ,  $t_{35}=2.27$ ,  $P<0.025$ ). Elevation did not affect the proportion of nectariferous flowers significantly for other inflorescence architectures.

## 2.5 Discussion

Only three studies have previously reported the occurrence of nectarless flowers in nectar-producing plants (Feinsinger, 1978; Thakar et al., 2003; Biernaskie and Cartar, 2004), leaving the impression that empty flowers occur rarely. In contrast, my survey detected empty flowers in 81% of the 52 species examined, indicating that empty flowers are prevalent in nature. Presumably the commonness of this phenomenon has largely been overlooked, even though nectar production is measured frequently in studies of plant reproduction, because empty flowers are considered as measurement errors, or the consequence of immature or old flowers. Instead, my results indicate that disregarding empty flowers provides an incomplete picture of nectar production, its function and influences. Rather, the occurrence of empty flowers should be embraced to understand flowering physiology and plant-pollinator relations.

The purpose of empty flowers has long been debated, with most discussion focusing on either empty flowers as a means of saving energy (Bell, 1986; Gilbert et al., 1991; Sakai, 1993) or of enhancing pollination (Thakar et al., 2003; Smithson and Gigord, 2003). The results of my survey suggest that empty flowers can serve both functions, although their relative importance seems to depend on a species' nectar production per flower. (Figure 2.10). For species that produce less than  $\sim 0.1$  mg sugar per flower the proportion of empty flowers increases with flower size (Figure 2.10a), but declines with increasing display size (Figure 2.10b). These results are consistent with energy savings, but not with pollination enhancement. In contrast, for species that produce more than  $\sim 0.1$  mg sugar per flower the proportion of empty flowers decreases with flower size (Figure 2.10a), but increases with display size (Figure 2.10b). These results are inconsistent with energy savings, but support pollination enhancement. Thus, the interacting effects of sugar production with flower and display size suggest

a transition in the primary purpose of empty flowers from energy saving for species that produce limited nectar to pollination enhancement for species that produce considerable nectar. The seemingly paradoxical lack of evidence for energy saving for species that produce considerable nectar seems to reflect the relative cost of nectar for these species. In particular, the proportional investment in nectar production declines with flower size among species (Figure 2.8), so that large-flowered species invest proportionally less total floral energy in nectar than small-flowered species.

Previous studies on the energy-saving advantages of empty flowers have argued that nectar production is costly (Bell, 1986; Gilbert et al., 1991; Sakai, 1993). Support for this assumption is drawn from Southwick's (1984) report that *Asclepias syriaca* invests 37% of the plant's total energy during flowering in nectar. In contrast, for the 52 species that I studied, estimates of nectar costs as a proportion of floral investment averaged only 3.3 ( $\pm 2.3$ )% (Figure 2.8), which is consistent with Harder and Barrett's (1992) results. In addition, floral photosynthesis in some species may completely offset the cost of nectar production (Zimmerman, 1988b). Thus, Southwick's (1984) findings for *A. syriaca* seem to be an exception, rather than the rule, perhaps because of the unusual requirements for pollen deposition in milkweeds (see Harder and Barrett, 1992). Nevertheless, my results indicate that the relatively high cost of nectar production in species that produce little nectar, which tend to be small-flowered, is sufficient that production of empty flowers may provide an energy saving.

My results also suggest that empty flowers may function to enhance pollination in species that produce considerable nectar per flower, which tend to be large-flowered. The positive influence of display size on the proportion of empty flowers for these species should allow species with large displays to encourage attracted pollinators to leave plants after relatively few flower visits, an expectation that I test in Chapter 3. This response should reduce the opportunity for self-pollination (de Jong et al.,

1993) and increase the proportion of pollen that successfully reaches the stigmas of conspecific plants (Harder and Thomson, 1989), as I address in Chapter 4.

Further support for the pollination-enhancement hypothesis can be found in the variation in the proportion of empty flowers among species with different inflorescence architectures. The high proportion of empty flowers in species with umbels and capitula architectures is consistent with Jordan and Harder's (2006) theoretical finding that such inflorescences are particularly susceptible to geitonogamy because of the high proportion of flowers visited by individual pollinators. Under these conditions, species with umbels and capitula would benefit most from having higher proportion of empty flowers to promote pollinators to leave plants early and carry pollen to other plants.

The primary function of empty flowers appears to follow a dichotomy. In small-flowered species that invest relatively heavily in sugar production, empty flowers seem to serve as an energy-saving strategy. However, in large-flowered species with large floral displays empty flowers may act primarily to manipulate pollinator behaviour and enhance pollination. Species that lie between these extremes may benefit more equally from energy saving and pollination enhancement.

Overall, this survey of nectarless flowers is the first comprehensive survey to demonstrate that empty flowers occur commonly in natural populations of nectar-producing species. Thus empty flowers are not exceptions, as suggested by previous reports in the literature, but instead are the rule. Furthermore, empty flowers probably function in both energy saving and pollination enhancement, depending on a species' flower size, mean nectar production and floral display size.



# Chapter 3

## Effects of Nectarless Flowers on Pollinator Behaviour

### 3.1 Introduction

Pollinator behaviour has strongly influenced the evolution of floral traits, because the objectives of pollinators and plants differ. Animal-pollinated plants rely on pollinators to transfer pollen and initiate outcrossing, a service provided incidentally by pollinators as they visit plants to obtain food for themselves and/or their offspring. In response, selection for improved pollen dispersal has resulted in the evolution of floral nectar production in most angiosperm clades, which serves both to attract pollinators and to control the duration of their visits (Keasar, 2000; Shafir et al., 2003). From a pollinator's perspective, access to abundant nectar is desirable, as it increases the animal's food intake rate (Heinrich and Raven, 1972). In contrast, production of abundant nectar can be disadvantageous for plants if nectar production is physiologically expensive and/or long visits to multiple flowers on the same individual increase self-pollination and reduce pollen export (see Chapter 2). Thus,

the evolution of nectar production probably implements a compromise between the benefits of pollinator attraction and mating costs.

Variable nectar production among a plant's flowers may provide one means of manipulating pollinator behavior to a plant's advantage (de Jong et al., 1993; Biernaskie and Cartar, 2004). Hodges (1981) found that bumble bees (*Bombus*) use nectar volume as a cue to decide when to depart plants to forage economically. In particular, bees remained on *Delphinium nelsoni* plants if floral-nectar volumes exceeded  $\geq 0.5 \mu\text{L}$ . This type of foraging is consistent with a threshold-volume departure rule, whereby bees remain on a plant if the volume in the current flower is above the bee's threshold (Hodges, 1985). This departure rule may depend on current nectar availability in the environment. In particular the marginal value theorem (Charnov, 1976) proposes that a pollinator should continue foraging on an inflorescence until its rate of energy return decreases to the average in the plant population. This average will depend on the interaction between mean nectar production rates and pollinator abundance. Plants in populations visited by relatively few pollinators should offer relatively abundant nectar, because of nectar accumulation during the long interval between successive pollinator visits, resulting in pollinators visiting more flowers per plant than in populations with many pollinators.

Subsequent studies of pollinator behaviour indicated that bees' departures from plants involve more complicated responses than expected from a simple threshold-departure rule. Biernaskie et al. (2002) demonstrated that bumble bees and hummingbirds can use variance-averse departure rules when foraging, in which pollinators visited only a proportion of available flowers when inflorescences differed in their nectar availability. Biernaskie et al. (2002) suggested that pollinators perceive variable patches as "risky" and attempt to avoid them. Bumble bees have also been suggested to use probabilistic departure rules, whereby the probability of departure

is a decreasing function of the amount of nectar received at the current flower (Cresswell, 1990; Kadmon and Shmida, 1992). Kadmon and Shmida (1992) suggested that departure rules depend on the plant's architecture and the nectar distribution within it. The threshold departure rule may be the best strategy when pollinators forage systematically from bottom to upper flowers and there is a decreasing nectar gradient up the inflorescence (i.e. on a spike or raceme inflorescences). Under these circumstances, nectar extracted from current flowers is a good predictor of the rewards to come. However when flower arrangements or nectar distribution show little consistency, rewards received from a current flower are not a good indication of further rewards. Under these circumstances, pollinators would forage most economically by using information from multiple flowers to decide when to leave an inflorescence, so that they do not leave an otherwise rewarding inflorescence after visiting a single unrewarding flower.

Production of a fraction of nectarless flowers may benefit plant reproduction by influencing pollinators directly. Pollinators spend less time visiting nectarless flowers and visit fewer flowers within an inflorescence (Cartar, 2004). For plants with large floral displays, visits to multiple flowers can be detrimental because of reduced pollen export associated with self-pollination (pollen discounting: de Jong et al., 1993). Furthermore, in self-compatible species, self-pollination may lead to inbreeding depression among offspring (Schemske and Lande, 1985; Charlesworth and Charlesworth, 1987b). Thus, plants that produce some empty flowers may realize the benefits of large displays through pollinator attraction while avoiding some of the associated mating costs.

In this chapter, I test several predictions concerning the influences of nectarless ("empty") flowers on the behaviour of bumble bees visiting *Chamerion angustifolium* (L.) Holub (Onagraceae). This species displays flowers in vertical racemes on which

bees typically forage from the bottom upwards (Galen and Plowright, 1985a). To examine the effects of empty flowers on bee behaviour I extracted the nectar from differing proportions of flowers and observed the responses of the first bee to visit manipulated inflorescences. If they forage using a threshold-volume departure rule, bees encountering an empty flower should probe relatively briefly and then leave the inflorescence in search of a more rewarding plant. Alternatively, if bees encounter few empty flowers, they should spend more time probing flowers and visit more flowers within an inflorescence. Based on the observed pollinator responses, I briefly consider the likely consequences of producing a fraction of empty flowers for plant mating.

## 3.2 Materials and Methods

### 3.2.1 Study Species and Sites

*Chamerion angustifolium*, or fireweed, is a holarctic herbaceous perennial, which commonly occupies recently disturbed habitats (Galen and Plowright, 1985b). The large, protandrous, purple-pink flowers of this species include eight anthers and a large four-lobed stigma. Flowers are arranged on a raceme, with typically 10-15 flowers open simultaneously (Moss, 1983). *Chamerion angustifolium* is pollinated primarily by large-bodied bees, especially bumble bees (*Bombus* sp.), which I considered exclusively.

I studied the responses of bumble bees to different proportions of empty *Chamerion angustifolium* flowers during 2004 and 2005 at four sites: three located in the Front Range of the Rocky Mountains in western Alberta (Kananaskis Country) and one in central British Columbia (Mt. Revelstoke National Park) (see Table 3.1 for details). The three Kananaskis populations (Beaver Ponds, Powderface and KFS)

are located in flat, open meadows, whereas the Skunk Cabbage population is located on a sloped moist grassland. Weather during the experiments was generally warm and dry (see Table 3.1). To characterize each population I measured the number of open flowers per inflorescence (display size: Beaver Ponds,  $n=110$ ; KFS and Skunk Cabbage,  $n=60$ ; Powderface,  $n=14$ ), the number of bees visiting individual inflorescences during 10-min observation periods (visitation rate: Beaver Ponds, KFS and Skunk Cabbage,  $n=20$ ; Powderface,  $n=40$ ), the number of flowers visited by individual bees per inflorescence (Beaver Ponds,  $n=20$ ; KFS and Skunk Cabbage,  $n=10$ ), the duration of pollinator visits to individual plants (Beaver Ponds,  $n=20$ ; KFS and Skunk Cabbage,  $n=10$ ), and the nectar standing crop (Beaver Ponds, KFS and Skunk Cabbage,  $n=20$ ; Powderface,  $n=40$ ). I did not measure the number of flowers visited or duration of pollinator visits to individual plants for the Powderface population.

To quantify 24-h nectar production per flower and its variation within *Chamerion angustifolium* inflorescences I sampled 11 plants (106 flowers) in the Beaver Ponds population during summer 2004. Each plant was covered with a nylon mesh bag to exclude pollinators and allow nectar replenishment of previously visited flowers. After 24 h, I recorded the position of each open flower, numbered from the top (youngest) to the bottom (oldest) flower. I then measured the flower's nectar volume with calibrated microcapillary tubes (Drummond, Broomall, PA, USA) and nectar concentration with a hand-held, sucrose refractometer that had been adjusted to measure small volumes (Bellingham and Stanley, Tunbridge Wells, UK). The measurements of nectar volume and concentration were used subsequently to calculate sugar production.

Table 3.1: Descriptions of *Chamerion angustifolium* populations studied in western Alberta ( $n=3$ ) and British Columbia ( $n=1$ ), including the sampling year, location, population size, mean daily maximum temperature ( $\pm$ SD) and mean daily precipitation ( $\pm$ SD). Weather data were obtained for the nearest Environment Canada weather station for the duration of each experiment.

Population	Year	Location	Pop. Size	Daily Maximum Temp. °C	Daily Precipitation (mm)
Beaver Ponds	2004	51° 03' N, 114° 54' W	468	21.8( $\pm$ 4.5)	5.78 ( $\pm$ 9.7)
KFS	2005	51° 01' N, 115° 02' W	120	25.8( $\pm$ 1.6)	0.0 ( $\pm$ 0.0)
Powderface	2004	51° 01' N, 114° 53' W	133	18.8( $\pm$ 4.5)	8.5 ( $\pm$ 13.3)
Skunk Cabbage	2005	51° 02' N, 118° 00' W	613	25.8( $\pm$ 1.6)	0.3 ( $\pm$ 0.3)

### 3.2.2 Experimental Design

To assess the effect of empty flowers on bumble-bee behaviour, I removed the nectar from 0%, 20%, 40%, 60%, 80%, or 100% of the flowers on randomly selected inflorescences in each population (Beaver Ponds,  $n=20$ ; KFS,  $n=10$ ; Skunk Cabbage,  $n=10$ ; Powderface,  $n=14$  for 20% only). I tested bee responses to one inflorescence at a time, with treatments applied in random order. For each inflorescence I chose the flowers to be emptied randomly, recorded their positions within the inflorescence, and removed their nectar with microcapillary tubes. I then observed the manipulated inflorescence until the next bee visit, which I recorded with a hand-held digital video camera (Sony DCR-TRV900).

I viewed the video-recording of each inflorescence visit twice to obtain necessary observations. Immediately after a bumble bee left a manipulated inflorescence, I viewed the video-recording and recorded the sequence of flower visits, so that I could associate visits with a flower's prior nectar status. Later, I analyzed the video-recording again frame by frame to measure the durations of each flower visit and the entire inflorescence visit (first to last contact) with an accuracy of 1/30 s.

Manipulation of nectar volumes could alter floral characteristics and so affect the behaviour of bees independently of their response to nectar availability (e.g. scent left by observer). To assess this possibility, I compared the time bees spent on individual flowers and the number of flowers visited for 13 unmanipulated inflorescences (inflorescence that were not touched) and 12 control inflorescences on which I probed flowers with a microcapillary tube, but did not remove nectar.

### 3.2.3 Statistical Analysis

General linear models (Kutner et al., 2005) were used to assess normally distributed responses. I used this approach to assess variation among populations (fixed factor) in characteristics that could influence bee behaviour, including inflorescence display size, total number of visits to an inflorescence, relative starting position of a pollinator within an inflorescence, number of flowers visited per inflorescence and the duration of inflorescence visits. The analysis of flowers visited per inflorescence also included display size as a continuous independent variable. Dependent variables were ln-transformed in the examination of the effects of empty flowers on bee behaviour and total foraging time within an inflorescence to normalize the distribution of residuals. General linear models were also used to assess differences between control treatments, 24-h nectar volume, concentration and sugar production, and nectar standing crop differences (volume and sugar). I used linear regression to examine variation in nectar volume, concentration and sugar with flower position within *Chamerion angustifolium* inflorescences for the Beaver Ponds population. Analysis of the duration of visits to individual flowers considered repeated measures for individual bees, so I used restricted maximum likelihood (Jennrich and Schluchter, 1986) to characterize the autoregressive covariance between each bee's successive responses. Denominator degrees of freedom for  $F$ -tests were calculated by the Kenward and Roger (1997) approximation, which can result in fractional degrees of freedom.

I used generalized linear models (McCullagh and Nelder, 1989) to assess the probability that a bee departed a *Chamerion angustifolium* inflorescence after individual flower visits (binary variable). This analysis considered a binomial error distribution and a logistic link function. Because this analysis considered responses by individual bees to multiple flowers per inflorescence, I used generalized estimating



equations and an autoregressive variance-covariance model to account for possible non-independence of repeated measures (Liang and Zeger, 1986). Independent variables initially considered in this analysis included the proportion of empty flowers within an inflorescence, the population, the plant's display size, the relative position of the flower within the inflorescence, the number of empty flowers already visited by the bee on the inflorescence, the total number of flowers already visited on the inflorescence, the time spent on the plant, and the duration of the flower visit. I used backward elimination based on score statistics to exclude nonsignificant independent variables ( $\alpha=0.05$ ).

To illustrate independent effects, I adjusted the observed response for individual observations to exclude the influences of all significant independent variables other than the predictor of interest. Details of this technique are described in section 2.3.2.

### 3.3 Results

#### 3.3.1 Nectar Production

Nectar production during 24 h varied strongly among flowers within the 11 inflorescences sampled in the Beaver Ponds population during 2004. All flowers produced some nectar, with volume ranging from 0.17 to 7.38  $\mu\text{L}$ , with a mean ( $\pm\text{SD}$ ) of 2.22 ( $\pm 1.41$ )  $\mu\text{L}$ . Nectar volume ( $V$ ) varied significantly with flower position ( $P$ ), generally increasing from the uppermost, youngest, male-phase flowers to the lowermost, oldest, female-phase flowers (Figure 3.1a:  $V=0.65+0.244P$ ,  $F_{1,104}=82.95$ ,  $P<0.0001$ ,  $R^2=0.44$ ). On average, flowers produced nectar with 38.2% ( $\pm 11.69$ ) sucrose equivalents, with a weak decline in concentration ( $C$ ) from upper to lower flowers (Figure 3.1b:  $C=44.01-0.91P$ ,  $F_{1,104}=10.29$ ,  $P<0.005$ ,  $R^2=0.09$ ). Overall,

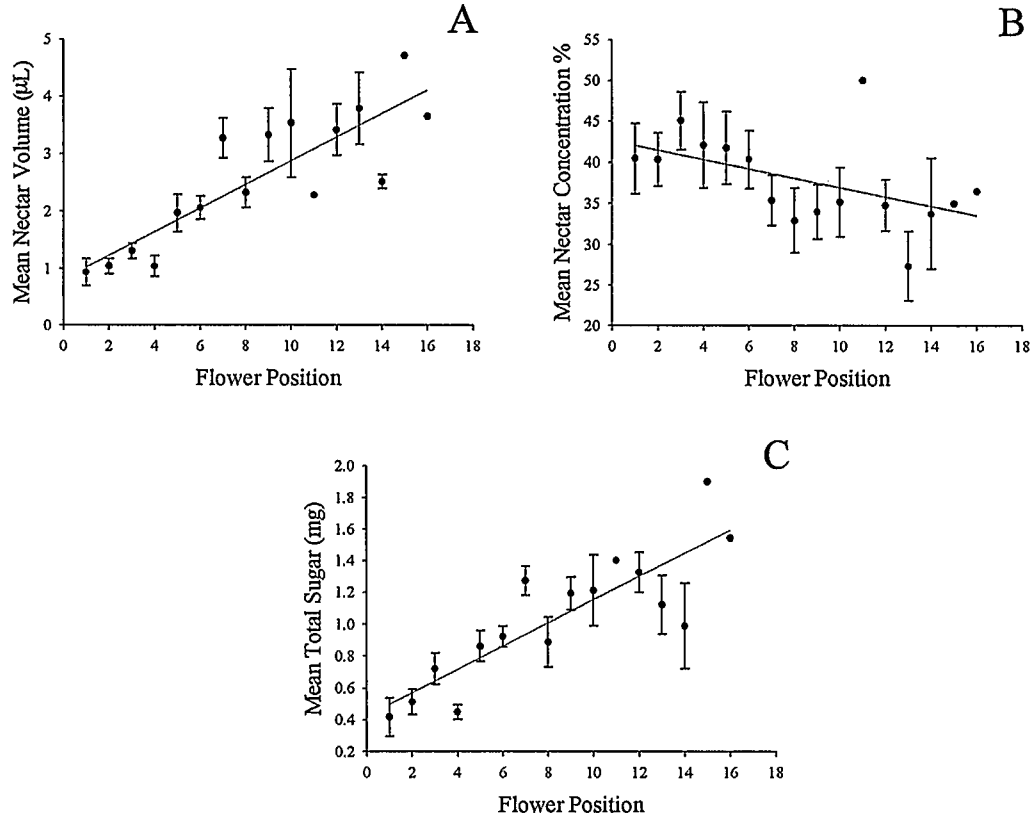


Figure 3.1: Relation of mean ( $\pm$ SE) a) nectar volume ( $\mu$ L), b) concentration (%) and c) sugar production (mg) to flower position in 11 *Chamerion angustifolium* inflorescences from the Beaver Ponds population. The upper-most flower on the inflorescence is designated as 1.

flowers produced  $0.89 (\pm 0.45)$  mg of sugar and sugar production ( $S$ ) increased from upper to lower flowers (Figure 3.1c:  $S = 0.42 + 0.07P$ ,  $F_{1,104} = 69.61$ ,  $P < 0.0001$ ,  $R^2 = 0.40$ ).

### 3.3.2 Population Characteristics

The four populations that I studied differed significantly in floral display size, bee abundance and aspects of bee behaviour. Overall, inflorescences displayed an

average ( $\pm$ SD) of 9.1 ( $\pm$ 2.37) flowers, but mean display size varied significantly among populations ( $F_{3,240}=3.54$ ,  $P<0.025$ ). In particular, display size did not differ significantly among the Alberta populations (Beaver Ponds, KFS and Powderface); however, they displayed fewer flowers per inflorescence than the British Columbia population (Skunk Cabbage) (based on Dunn-Šidák multiple comparisons: Figure 3.2a).

The three measured aspects of bee behaviour also differed among the study populations. Bee abundance apparently varied among populations, as indicated by significant differences in visitation rates to inflorescences during 10-min observation periods ( $F_{3,49}=13.78$ ,  $P<0.0001$ ). Plants in the British Columbia population experienced much more frequent visitation than those in the Alberta populations, which did not differ among themselves (Figure 3.2c). Bees visited differing numbers of flowers per inflorescence among the study populations ( $F_{2,37}=3.70$ ,  $P<0.05$ ), visiting significantly fewer flowers in both the Beaver Ponds and KFS population than in the Skunk Cabbage population (Figure 3.2b). The total duration of inflorescence visits differed among populations ( $F_{3,240}=2.73$ ,  $P<0.05$ ), with briefer visits at the Beaver Ponds and KFS populations than at the Powderface and Skunk Cabbage populations.

During the nectar-manipulation experiments, flowers in the Alberta populations offered considerably less sugar than those in the British Columbia population ( $F_{3,96}=86.67$ ,  $P<0.0001$ : see Figure 3.2d). Given that bees visited inflorescences more frequently in the British Columbia population (Figure 3.2c), this result suggests that plants in this population produced nectar more rapidly than those in the Alberta populations.

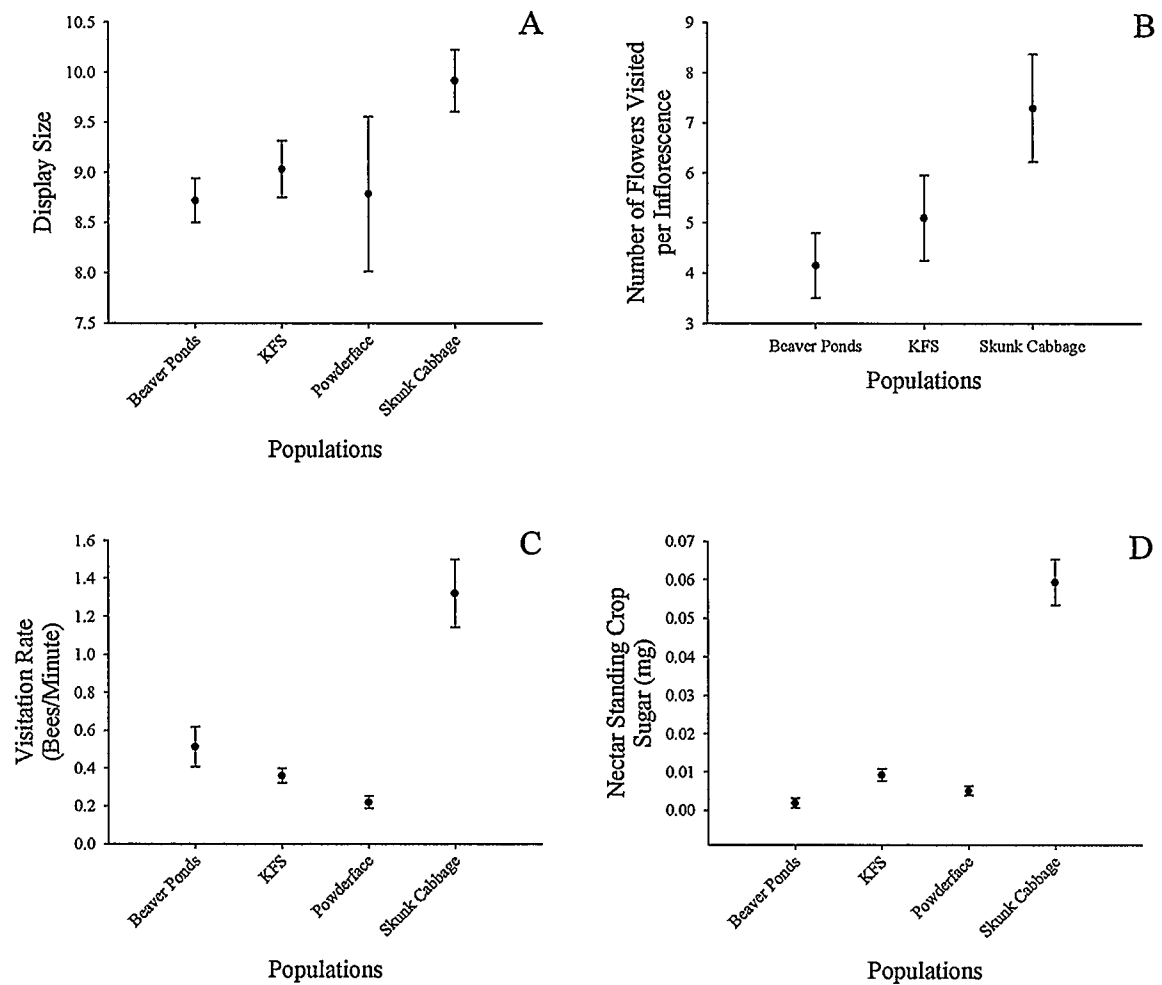


Figure 3.2: Comparison of mean ( $\pm$ SE) a) display size, b) number of flowers visited by bees per inflorescence, c) visitation rate and d) sugar standing crop for four *Chamerion angustifolium* populations (Beaver Ponds, KFS, Powderface, Skunk Cabbage).

### 3.3.3 Unintended Effects of Nectar Manipulation

The manipulation of flowers to extract nectar did not affect bee behaviour significantly. Bees visited equivalent numbers of flowers on unmanipulated and control inflorescences ( $F_{1,23}=1.74$ ,  $P>0.10$ ). Furthermore, the duration of visits to individual flowers and inflorescences did not differ between unmanipulated and control inflorescences ( $F_{1,23}=0.01$ ,  $P>0.75$ ;  $F_{1,23}=1.26$ ,  $P>0.25$ , respectively).

### 3.3.4 Effects of Empty Flowers on Bee Behaviour

#### Bumble-bee Starting Position

The presence of empty flowers in *Chamerion angustifolium* inflorescences did not affect the flower position within inflorescences at which bees began foraging ( $F_{1,242}=3.80$ ,  $P>0.05$ ). Of the variables examined, only the number of open flowers affected bees' starting positions significantly ( $F_{1,240}=16.73$ ,  $P<0.001$ ,  $R^2=0.07$ ). Specifically, bees visiting small displays tended to begin half-way up inflorescences (50%), whereas bees usually began foraging about a quarter to a third of the way up larger inflorescences (Figure 3.3).

#### Influences on Departure

The probability that a bee departed an inflorescence after visiting a specific flower depended on a variety of influences (Table 3.2). Departure probability differed significantly among study sites, with the bees that visited the Skunk Cabbage population exhibiting the lowest departure probability (Figure 3.4a), which resulted in bees visiting more flowers per inflorescence (Figure 3.2b). A bee's relative position on an inflorescence also influenced its departure probability, as bees were more likely to depart inflorescences from upper flowers than from lower flowers (Figure 3.4b). This

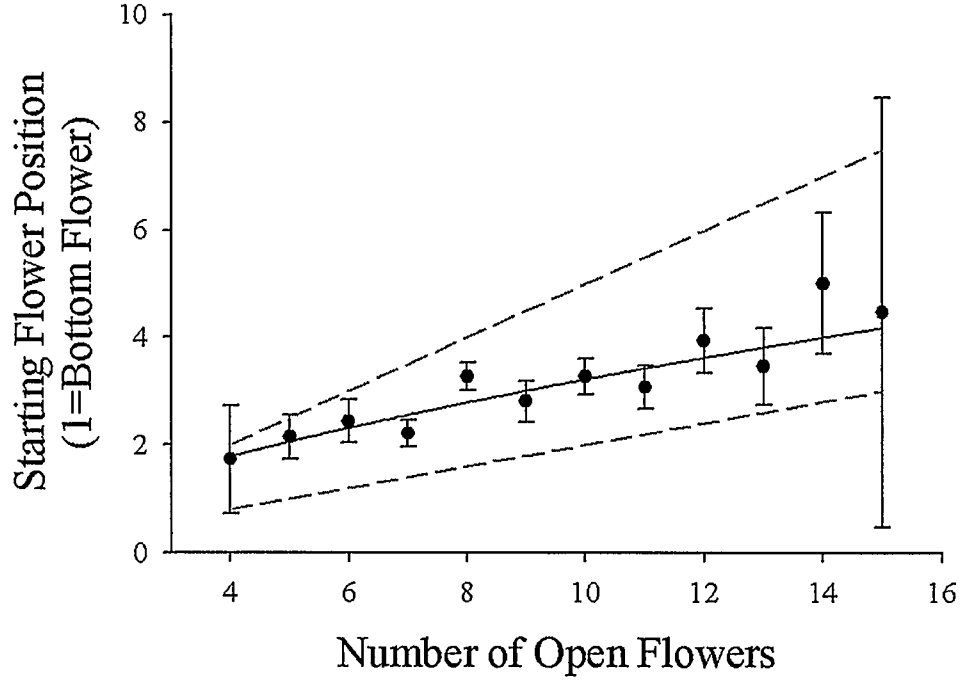


Figure 3.3: Relation of the mean( $\pm$ SE) starting position of bumble bees (*Bombus* sp.) on *Chamerion angustifolium* inflorescences to display size. The solid line depicts the least-squares regression line, whereas the dotted lines illustrate starting positions in the lower 20% and 50% of inflorescences.

result is not surprising as bees tend to move upward on *Chamerion angustifolium* inflorescences (Routley and Husband, 2003). In general, bees were more likely to leave flowers on inflorescences with high proportions of empty flowers; however, this effect was evident only on inflorescences with >40% empty flowers (Figure 3.4c). A bee's departure probability tended to increase as it encountered more empty flowers, but visits to at least four empty flowers were needed to stimulate this response (Figure 3.4d). As a result, the nectar state of the last-visited flower (nectarless or rewarding) did not significantly influence a bee's departure probability ( $T_1=2.66$ ,  $P>0.10$ ).

Table 3.2: Results of a generalized linear model considering the influences on bees' departures from *Chamerion angustifolium* inflorescences.

Characteristic	<i>df</i>	<i>T</i>	<i>P</i>
Proportion of Empty Flowers	5	27.47	<0.0001
Relative Position within an Inflorescence	1	33.72	<0.0001
Number of Empty Flowers Encountered	1	13.62	<0.005
Location	3	15.66	<0.005

### Foraging Duration on Inflorescences and Flowers

The duration of bumble-bee visits to inflorescences varied significantly with features of both the inflorescence and the bee ( $R^2=0.29$ ). As expected, visit duration decreased linearly with an increasing proportion of empty flowers (Figure 3.5a:  $F=16.61$ ,  $df=5$ ,  $P<0.0001$ ). In contrast, bees spent longer foraging for nectar on large inflorescences (Figure 3.5b: partial regression coefficient  $b \pm SE=0.108 \pm 0.027$ ,  $F=9.39$ ,  $df=1$ ,  $P<0.0001$ ). Finally, bumble bees that began foraging high on an inflorescence spent more time foraging on that inflorescence (Figure 3.5c:  $b \pm SE=0.920 \pm 0.229$ ,  $F=16.20$ ,  $df=1$ ,  $P<0.0001$ ).

Two factors affected the duration of visits to individual flowers significantly. Visit duration differed among populations ( $F_{3,240}=3.72$ ,  $P<0.05$ ), with bees visiting flowers significantly longer in the KFS and Skunk Cabbage populations than in the Beaver Ponds and Powderface populations ( $F_{1,240}=7.52$ ,  $P<0.01$ ). The duration of flower visits depended most strongly on a flower's nectar state ( $F_{1,116}=80.54$ ,  $P<0.0001$ ), with bees spending about 50% longer visiting flowers with nectar than empty flowers (Figure 3.6b).

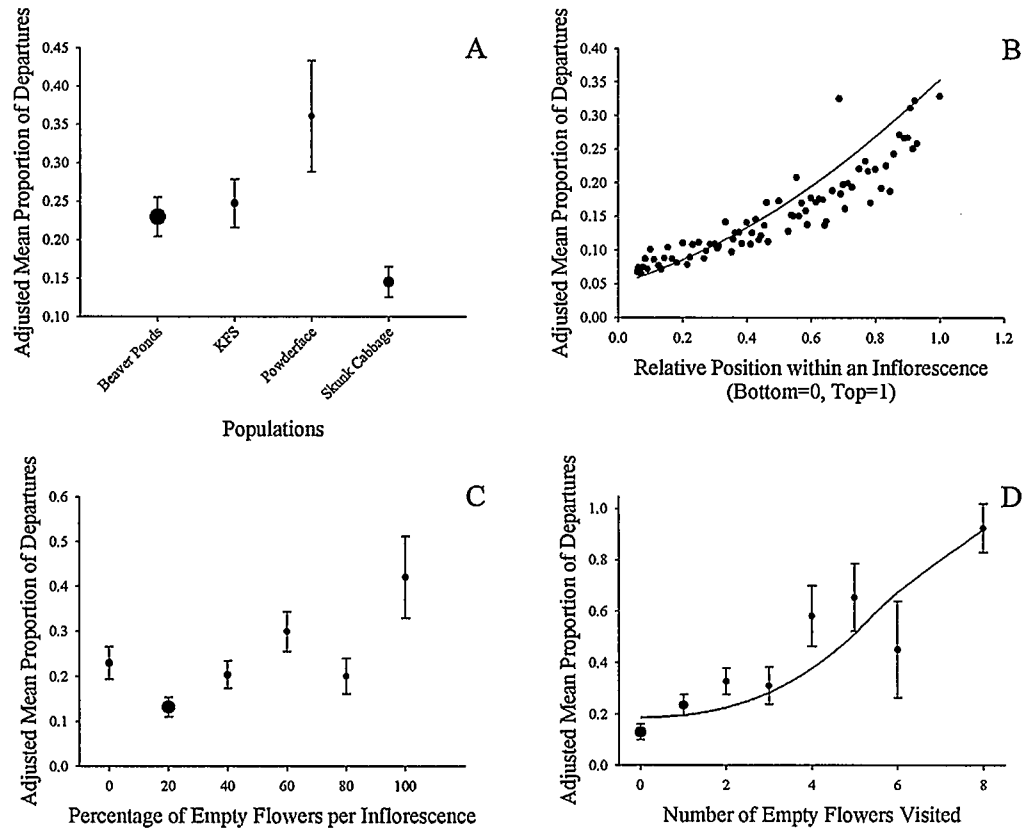


Figure 3.4: Adjusted mean ( $\pm$ SE) proportion of departures by bumble bees from *Chamerion angustifolium* inflorescences in relation to a) study population, b) a bee's relative position within an inflorescence c) the proportion of empty flowers within the inflorescence and d) the number of empty flowers that the bee had already visited on an inflorescence. Symbol size indicates relative sample size.



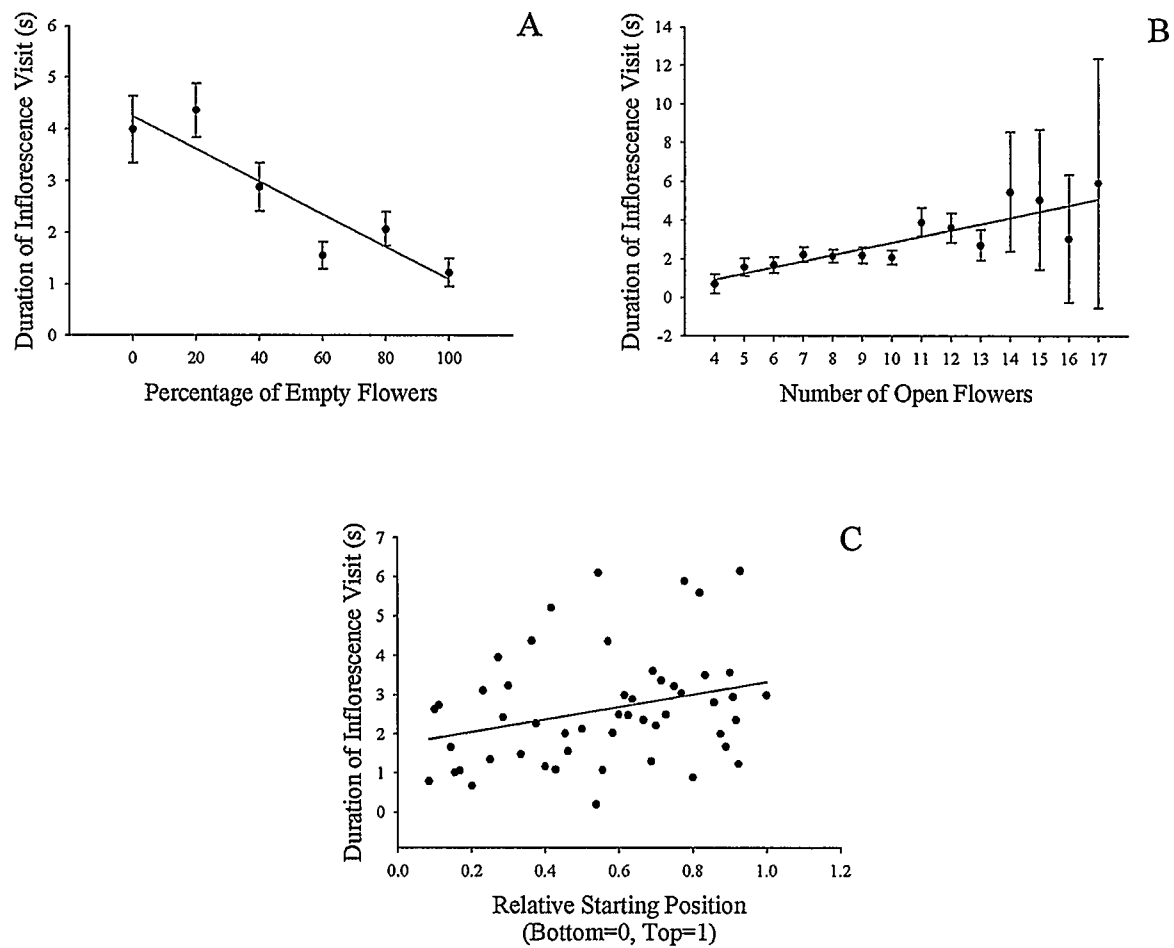


Figure 3.5: Influences of total foraging time of bumble bees on *Chamerion angustifolium* inflorescences, including a) the proportion of empty flowers, b) display size, and c) the bee's relative starting position. Error bars in a) and b) represent standard errors.

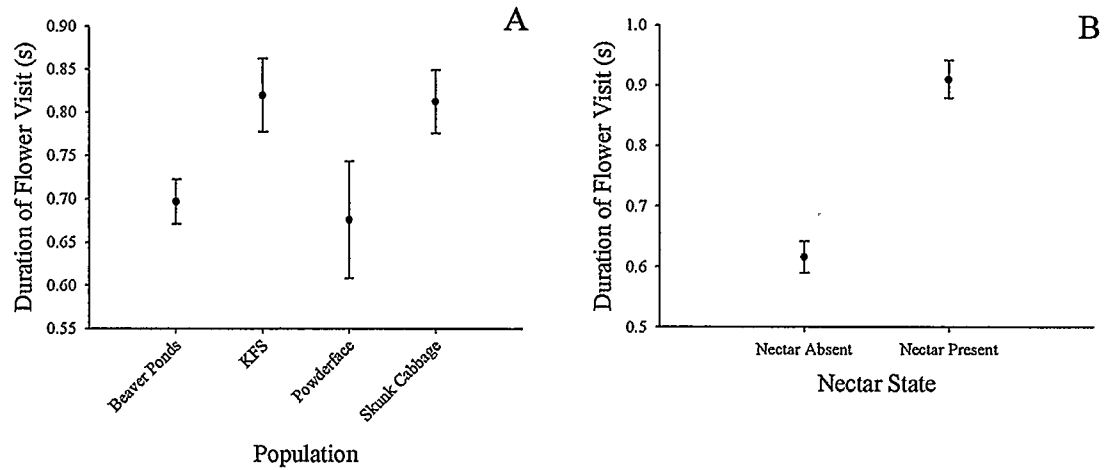


Figure 3.6: Influences on the mean ( $\pm$ SE) duration of visits to individual *Chamerion angustifolium* flowers by bumble bees, including a) population and b) the flower's nectar state.

## 3.4 Discussion

### 3.4.1 Responses to Nectarless Flowers

Nectarless flowers strongly influence bumble-bee behaviour within inflorescences. The probability of departure increased with an increasing proportion of empty flowers within an inflorescence (Figure 3.4a), which in turn reduced the time spent on inflorescences with empty flowers (Figure 3.5a). Interestingly, the effect of empty flowers did not interact significantly with any other measured variables, so that they influence bee behaviour independently measured of other plant characteristics.

Generally, pollinators spend less time on flowers that contain little or no nectar (Montgomerie, 1984; Kadmon and Shmida, 1992; Cresswell, 1999) and visit fewer flowers on poorly rewarding inflorescences (Hodges, 1995; Cartar, 2004). Brief visits to individual flowers with little or no nectar can result because less time is required to ingest the available nectar compared to more rewarding flowers (Harder, 1983;

Cresswell, 1999). Alternatively, pollinators foraging in an environment with poorly rewarding flowers, may develop a learned expectation of low nectar rewards and leave a flower or plant (Cresswell, 1999; Ferdy and Smithson, 2002).

Although bees departed *Chamerion angustifolium* inflorescences with a high proportion of empty flowers earlier than those with few empty flowers, they did not employ a threshold departure rule, as proposed by Hodges (1985; also see Pyke, 1978a; Best and Bierzychudek, 1982). In particular, the nectar state of the last flower visited by a bumble bee did not determine whether the bee left the plant. Instead, only  $\approx 18\%$  of visits to empty flowers were followed by a bumble bee departing an inflorescence. This result occurred even though *C. angustifolium* presents vertical inflorescences, on which bees generally visited from lower flowers upward. This foraging pattern should promote the development of positive correlations in nectar standing crop among flowers within an inflorescence, so that experience with one flower provides information about nectar availability in higher flowers (Kadmon and Shmida, 1992).

Positive relations of a bee's departure probability with both the proportion of empty flowers in an inflorescence (Figure 3.4c) and the number of empty flowers that the bee had already visited (Figure 3.4d) suggest that bumble bees foraged according to a probabilistic departure rule, and that the departure probability is a decreasing function of the amount of nectar received (Cresswell, 1990; Kadmon and Shmida, 1992; Collevatti et al., 1997). Thus, departure decisions were apparently based on integration of nectar information from more than one flower (also see Hartling and Plowright, 1979; Kadmon and Shmida, 1992). Foraging using a probabilistic departure rule is probably beneficial, because bees remain on highly rewarding plants with a few empty flowers, but depart early from plants with many empty flowers (Cresswell, 1990). In contrast, pollinators that respond to the nectar reward of the current flower (i.e. threshold departure rule) would often leave a highly rewarding

plant prematurely if by chance one of the first few flowers probed was empty and did not represent the actual rewards available within an inflorescence accurately.

Why did bees visiting *Chamerion angustifolium* inflorescences not use a threshold-departure rule, even though all flowers produce nectar? The explanation probably lies in the frequency with which flowers experienced bee visits in the populations that I studied. Based on the average visitation rate to inflorescences and the proportion of flowers visited per inflorescence for each population, the average time between visits for an individual flower ranged between 1-4.5 minutes (Beaver Ponds=3.5 min, KFS=4.5 min, Skunk Cabbage=1.0 min). Such brief intervisit intervals allow little time for nectar replenishment. Indeed, based on the standing sugar content (Figure 3.2d) and an average concentration of 40% sucrose (Figure 3.1b), flowers in the Alberta populations offered only about 0.01  $\mu\text{L}$  of nectar and those in the Skunk Cabbage population offered about 0.13  $\mu\text{L}$ . Thus, because of the frequent visitation in these populations, bumble bees probably commonly encountered empty flowers, which had just been visited by another bee. Such a nectar environment would create uncertainty for a bee in determining the reward status of an inflorescence. Under these conditions bees would forage more efficiently by integrating information from multiple flowers before deciding to leave a plant. This reliance on confirmation of a plant's nectar status from visits to several flowers is consistent with the relative insensitivity of bees visiting *C. angustifolium* to encountering up to 40% empty flowers within an inflorescence (Figure 3.4c).

### 3.4.2 Consequences for Plant Reproduction

The response of pollinators to empty flowers offers fitness benefits to the plant through pollen exchange and reproduction. Pollinators spent less time on empty flowers and

visited fewer flowers within an inflorescence with increasing proportions of empty flowers (also see Galen and Plowright, 1985b; Cresswell, 1990; Golubov et al., 1999). Responses to empty flowers often result in pollinators visiting only a small fraction of flowers within a plant, particularly when visitation rates are high (Augsburger, 1980; Robertson, 1992; Harder et al., 2004). In *Chamerion angustifolium* with high visitation rates, the percentage of flowers visited on an inflorescence ranged between 50-75% (Beaver Ponds=50%, KFS=60% and Skunk Cabbage=75%). Decreases in flower visits and visit duration with increasing proportions of empty flowers reduces the proportion of pollen removed by a single pollinator, minimizing pollen loss (removal or transit loss: Harder and Thomson, 1989) and reducing geitonogamy and its deleterious effects on plant reproduction, primarily through inbreeding depression (de Jong et al., 1993).

Despite the benefits of pollination enhancement demonstrated by a pollinator's response to empty flowers, no empty flowers were found in inflorescences of *Chamerion angustifolium* (Chapter 2). This paradox can be explained by recognizing that empty flowers can occur in two ways: inhibition or cessation of nectar production by the plant, or recent nectar extraction by a pollinator. Species with high visitation rates, like *Chamerion angustifolium*, likely do not need to inhibit nectar production, because frequent visits by bees create a large proportion of empty flowers. This circumstance would be particularly true for *C. angustifolium*, a species that begins flowering during mid to late July. In the areas sampled, most other plant species have already flowered, lowering the diversity of flowering species and limiting a pollinator's choice of nectar sources. This combination of low flowering diversity and maximal pollinator abundance results in frequent visitation to *Chamerion angustifolium*, even though its nectar standing crop is very low, and pollinators produce a high frequency of empty flowers.

Overall, this study demonstrated how pollinators (bumble bees) react to different proportions of empty flowers. Increases in the proportion of empty flowers decreased the number of flowers visited and the time spent on each flower, causing an overall increase in the probability of a pollinator's departure from a plant. Interestingly, a flower's nectar state did not significantly influence a pollinator's decision to leave an inflorescence. Instead, pollinators appear to forage using a probabilistic departure rule, allowing them to integrate information about the states of multiple flowers when deciding to remain or leave a plant. Ultimately, plants with high proportions of empty flowers, regardless of how they are produced (inhibition of nectar production or through visitation), should have increased reproductive success by limiting the amount of pollen removed by a individual pollinators, thereby minimizing diminishing returns associated pollen loss and self-pollination. These mating consequences are examined further in Chapter 4.

# Chapter 4

## Pollen Transfer

### 4.1 Introduction

A plant's pollination performance depends on three factors: the number of pollinator visits; the number of flowers visited by each pollinator; and the effectiveness of pollinators in transferring pollen (Cresswell, 1999). Nectar influences all three aspects of pollination, and so can contribute significantly to the incidence of self-pollination and a plant's pollen import and export. Specifically, nectar attracts pollinators to forage on a plant's flowers and to return for subsequent foraging (Real and Rathcke, 1991), it influences a pollinator's decision to continue foraging on the same plant or to leave in search of better rewards (Pyke, 1978a; Galen and Plowright, 1985b, Chapter 3), and it determines a pollinator's visit duration on an individual flower (Keasar, 2000; Shafir et al., 2003, Chapter 3). Longer visits increase pollen transfer (perhaps including self-pollination) by increasing the chance and extent of contact between a flower's sexual organs and a pollinator (Galen and Stanton, 1989; Murcia, 1990). Plants that consistently produce more nectar and/or display more flowers than the population average receive more pollinator visits, during which pollinators probe

more flowers and visit each flower longer (Zimmerman and Pyke, 1988b; Cresswell, 1999). The positive relation between nectar quantity and pollinator attraction increases pollen removal, influencing reproductive success through pollen dispersal (Harder and Thomson, 1989; Barrett et al., 1994). However increased visitation and visit duration often lower reproductive success through sub-optimal pollen transfer (e.g. pollen loss) and self-pollination within flowers (autogamy) or between flowers (geitonogamy) (Thomson and Plowright, 1980; Harder and Thomson, 1989; Johnson and Nilsson, 1999; Johnson et al., 2004).

Pollinators can remove over 80% of the pollen available in a flower during a single visit (Harder, 1990); however only about 0.5–1% reaches stigmas on other plants (Levin and Berube, 1972; Harder and Thomson, 1989; Harder, 2000). Harder and Thomson (1989) suggested that optimal pollen transfer occurs through numerous pollinator visits to a plant, each removing a small proportion of pollen. This outcome arises if increased removal by individual pollinators decreases the proportion of removed pollen that reaches stigmas, perhaps because of increased transport loss or self-pollination between flowers. Consequently, the probability of successful pollen transfer increases as plants limit the amount of pollen removed by individual pollinators, as long as enough pollinators visit to remove all of a flower's pollen.

Plants can restrict pollen removal through two general mechanisms: by controlling pollen production and availability, or by manipulating pollinator behaviour through floral traits. Pollen production and availability can limit pollen removal through packaging mechanisms that stagger pollen presentation (e.g. sequential anther dehiscence: Harder and Thomson, 1989), dispensing mechanisms that limit pollen removal by individual pollinators, or by increased display size, which increases the number of visits and time spent on a plant (de Jong et al., 1992; Ohashi and Yahara, 2001).



Most pollinators visit flowers for nectar (Simpson and Neff, 1983; Stpiczyńska, 2003a), so that altering nectar production or quantity can strongly influence plant reproduction and fitness. Pollinators forage to maximize their net energy intake, and so are sensitive to variation in nectar quantity within a species (Real, 1981; Biernaskie et al., 2002; Biernaskie and Cartar, 2004). Hodges (1985) and Dreisig (1989) proposed that pollinators forage on vertical inflorescences using a threshold departure rule and remain foraging on a plant until they encounter flowers that contain less than a threshold nectar volume, when they switch to a different inflorescence or plant. This response is also a feature of the marginal-value theorem (Charnov, 1976; Charnov and Bull, 1985), which proposes that a consumer forages on a resource patch until its rate of energy return decreases to that of the average patch. When flowers in a population contain little nectar on average, pollinators should adopt a low threshold, resulting in little motivation for choosing one flower over another (Harder, 1988). Alternatively, abundant nectar within a population should result in higher pollinator discrimination.

Many plant species produce some nectarless (empty) flowers (Chapter 2), which necessarily offer lower return than a pollinator's departure threshold for any rewarding plant species. Consequently, empty flowers should alter pollinators' behaviour, stimulating them to leave a flower, inflorescence or plant in search of better rewards. Such earlier departure than expected in the absence of empty flowers should affect a plant's reproductive success and fitness. The pollination-enhancement hypothesis proposes that empty flowers enhance pollination directly (see Chapter 2). Large floral displays promote pollinator attraction (Ohashi and Yahara, 2001), leading to geitonogamy and reduced fitness through inbreeding depression and pollen discounting (de Jong et al., 1992; Harder and Barrett, 1995). Inflorescences with some empty flowers may encourage pollinators to leave the plant early, thus reducing

geitonogamy and enhancing pollen export. With few pollinators, pollen removal is limited, so empty flowers should be few or non-existent to ensure pollination and pollen removal (a bet hedging strategy: Harder and Barrett, 1992). If pollinators are moderately abundant, empty flowers would promote efficient pollen export by encouraging pollinators to leave early, thus allowing several pollinators to remove fractions of available pollen (Harder and Thomson, 1989). Empty flowers will be most effective as a means of deceiving pollinators if not all flowers are empty. Pollinators would learn to avoid completely rewardless plants, resulting in low visitation and reproductive success (Smithson and Gigord, 2003). Alternatively, completely rewarding plants do not benefit from either energy savings or reduced self-pollination.

The effect of empty flowers on pollen transfer and empty flowers has received increasing recent attention; however most studies have considered completely rewardless species (Ackerman et al., 1994; Neiland and Wilcock, 1998; Johnson and Nilsson, 1999; Ferdy and Smithson, 2002; Johnson et al., 2003a). No study has examined the effects of differing proportions of empty flowers within inflorescences on pollen transfer. Furthermore, examinations of pollen transfer have primarily involved theoretical models and artificial array experiments (Iwasa et al., 1995; Baum and Grant, 2001; Ferdy and Smithson, 2002; Smithson and Gigord, 2003; however see Jersáková and Johnson, 2006). Therefore, I examined the effects of different proportions of empty flowers for self-pollination and pollen export in the tall white bog orchid (*Platanthera dilatata*). If empty flowers stimulate pollinators to leave a plant, the incidence of self-pollination should vary negatively with the proportion of empty flowers. As a result, plants with few empty flowers should export less pollen than those with many empty flowers, as long as pollinators continue visiting plants and removing pollen.

## 4.2 Materials and Methods

### 4.2.1 Study Species

*Platanthera dilatata* (Pursh) Lindley ex Beck (Orchidaceae), the tall white bog orchid, is a slender perennial herb between 15–70 cm tall, which typically occupies moist woods, bogs and meadows from Alaska to Newfoundland and south to Washington, New Mexico and New England (Brown, 2003). Each reproductive plant produces one inflorescence per flowering season, with typically 5–30 flowers (up to 80 flowers) arranged in a spike. The fragrant white flowers, which are pollinated by Lepidoptera, mostly moths (Boland, 1993), include an upper hood, a lower broad-based lip and a slender curved spur (Figure 4.1a). Flowering occurs from early June to mid-August, depending on location.

Orchid pollination differs from that of most angiosperms, because most orchids produce their pollen in aggregations (pollinia), which are removed as a unit (van der Pijl and Dodson, 1966; Dressler, 1981), rather than as independent grains. In derived orchid clades the pollinium is attached by a stipe or caudicle to a sticky viscidium, which adheres strongly to a pollinator's body. A *Platanthera dilatata* flower produces two of these aggregate structures, or pollinaria. While visiting a *P. dilatata* flower, a moth extends its proboscis into the nectar spur to drink nectar, and if its proboscis contacts the viscidium the attached pollinium is removed from its anther sac when the moth leaves the flower. Like 11% of orchid species, *P. dilatata* produces sectile pollinia (Dressler, 1993), which are removed as one unit, but are deposited on stigmas as smaller sub-units, known as massulae (Figure 4.1b). Thus pollen from a single pollinium can disperse to the stigmas of multiple flowers.

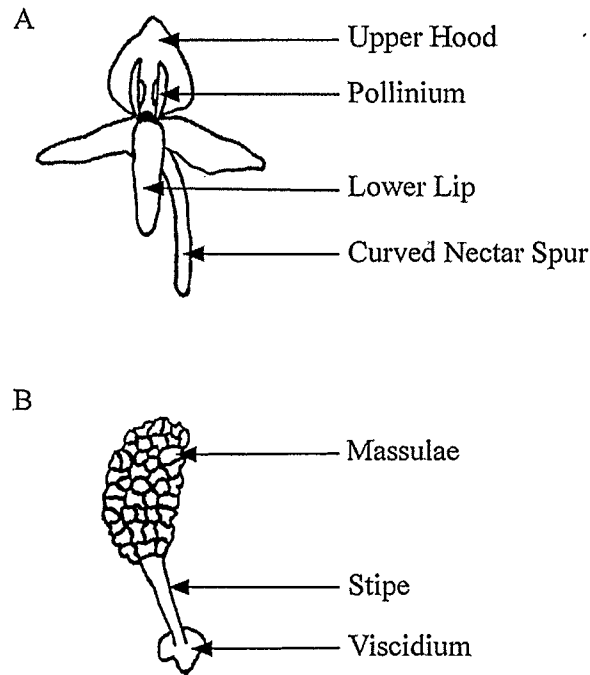


Figure 4.1: A diagram of a) the flower of *Platanthera dilatata*, adapted from Maad and Nilsson (2004) and b) the structure of a pollinarium, from Lukasiewicz (1999).

#### 4.2.2 Study Sites

I studied the pollination of *Platanthera dilatata* at seven sites in eastern British Columbia and one site in western Alberta (Table 4.1). All study sites were examined between July 6–26, and ranged in day-time maximum temperatures ( $\pm$ SD) from 12.3 ( $\pm$ 3.58) $^{\circ}$ C and a high of 24.3 ( $\pm$ 4.02) $^{\circ}$ C. The average population density was 1.11 plants/m<sup>2</sup>, with Takkakaw Falls Meadow being least dense (0.08 plants/m<sup>2</sup>) and Crowfoot Glacier being the most dense (6.72 plants/m<sup>2</sup>; Table 4.1).

Table 4.1: Locations of *Platanthera dilatata* populations studied in British Columbia ( $n=7$ ) and Alberta ( $n=1$ ) and the total number of plots examined. \*- denotes estimates were taken using quadrats.

Populations	# of Plots	Location	Elevation(m)	Pop. Size	Pop. Density (m <sup>2</sup> )
Bostock	8	51° 13' 41N, 117° 40' 16W	964	211	0.24
Bostock Railway	1	51° 13' 39N, 117° 40' 13W	960	24	0.54
Crowfoot Glacier	4	51° 39' 18N, 116° 24' 15W	1939	9681*	6.72*
Glacier Hwy	3	51° 13' 60N, 117° 42' 14W	998	598	0.40
Takkakaw Falls Meadow	2	51° 29' 44N, 116° 29' 03W	1515	122	0.08
Takkakaw Falls Roadside	3	51° 29' 26N, 116° 28' 48W	1504	322	0.48
Takkakaw Falls Stream	2	51° 29' 36N, 116° 28' 57W	1512	61	0.48
Yoho Hwy	3	51° 22' 04N, 116° 31' 16W	1273	62	0.28

### 4.2.3 Study Design

I characterized the variation in 24-h nectar production within and among *Platanthera dilatata* inflorescences in the Bostock population. Nectar volume was determined using 1  $\mu$ L capillary tubes and concentration was measured with a Bellingham and Stanley sugar refractometer that had been adjusted to accommodate small volumes. A total of 100 flowers were examined to quantify nectar patterns with a maximum of flowers used from each of 10 plants. Because most plants displayed more than 10 flowers, I sub-divided the inflorescence into five sections and selected two flowers randomly from each section to sample nectar.

The influence of empty flowers on pollen transfer was examined in all eight populations. Within each population, I selected groups of four plants (plots) on which I stained the pollinia of all flowers and examined the incidences of subsequent pollinarium removal, self-pollination and pollen export within the surrounding 20 m. The location of each plot was chosen to maximize the number of plots for a population, with no overlap in export area. To increase the proportion of empty flowers on inflorescences, I randomly selected 0%, 33%, 66% or 100% of the flowers on an inflorescence and emptied them using filter-paper wicks. To remove the nectar I slit the nectar spur along approximately two-thirds of its length from the base with a scalpel and then probed to the tip with a filter-paper wick. The tip of the spur was left intact to avoid adverse pollinator behaviour and unusual moth-flower contact caused by a moth pushing its proboscis through the spur.

To assess pollination outcomes for manipulated inflorescences, I labeled all pollinia on the 98 manipulated inflorescences with a histochemical stain injected into the anther sac (Peakall, 1989). Flowers of each treatment received the same stain, which differed from that applied to other treatments within a population. The stains used

were trypan red (2%), brilliant green (1% w/v), methylene aniline blue (1%) and Bismark brown (1%), for 0, 33, 66 and 100% empty flowers, respectively (Peakall, 1989).

Treatments were applied approximately 1 h before dusk and left overnight to allow pollinators to visit inflorescences and disperse massulae within the population. The following day I examined each donor plant to count the pollinaria removed during the preceding night and whether stained massulae had been deposited on its own stigmas (self-pollination). I also examined the stigmas of the other three stained plants in a group and other plants within 20 m of a stained donor for evidence of the donor's pollen (pollen export). If the 20-m neighborhood contained many unstained plants, I examined only 10 unstained individuals, so that the number of potential recipients examined could not exceed 13, including the other three stained plants within a group. When I found stained massulae on the stigma of an unstained plant, I measured the distance between the donor and recipient.

#### 4.2.4 Statistical Analysis

I used generalized linear models (McCullagh and Nelder, 1989) to assess the pollination outcomes of manipulating the proportion of empty flowers, which tested for significant effects with likelihood-ratio (G) tests. These analyses considered error distributions that reflected the characteristics of the observations (proportions - binomial distribution; exported massulae - Poisson distribution) and appropriate transformation (link function) of the dependent variables to linearize their relations to independent variables (proportions - logit transformation; massulae export - ln transformation).

All of these analyses included population, plot within population, and the

proportion of manipulated empty flowers as categorical independent variables. In addition, the analysis of pollinium removal considered both the number of open flowers displayed by a plant and the number of available pollinia as covariates. After identifying a significant categorical factor, I used Dunn-Šidák multiple comparisons to test specific differences between treatments or groups of treatments (Zar, 1999).

To illustrate independent effects, I adjusted the observed response for individual observations to exclude the influences of all significant independent variables other than the predictor of interest. Details of this technique are presented in section 2.3.2.

## 4.3 Results

### 4.3.1 Nectar Patterns in *Platanthera dilatata* Inflorescences

Plants in the Bostock population, where I quantified nectar availability, displayed an average ( $\pm$ SD) of 32.3 ( $\pm$ 17.20) open flowers per inflorescence. I observed three unidentified species of noctuid moths (Noctuidae) pollinating *Platanthera dilatata* in this population, with most pollinators visiting at dusk. The percentage of empty flowers per plant ranged from 0 to 30%, with an mean ( $\pm$ SD) of 10 ( $\pm$ 10.5)%. Flowers with nectar contained a mean ( $\pm$ SD) of 0.48 ( $\pm$ 0.31)  $\mu$ L with a mean concentration of 11 ( $\pm$ 3.99)%, so that the average flower contained 0.05 ( $\pm$ 0.04) mg of sugar.

A repeated-measures ANOVA (for plant) found that volume did not vary significantly with any plant characteristic ( $F_{1,98}=2.02$ ,  $P>0.10$ ). In contrast, nectar concentration and sugar amount declined with increasing display size ( $F_{1,98}=15.55$ ,  $P<0.005$ ;  $F_{1,98}=4.44$ ,  $P<0.05$ , respectively; see Figure 4.2).



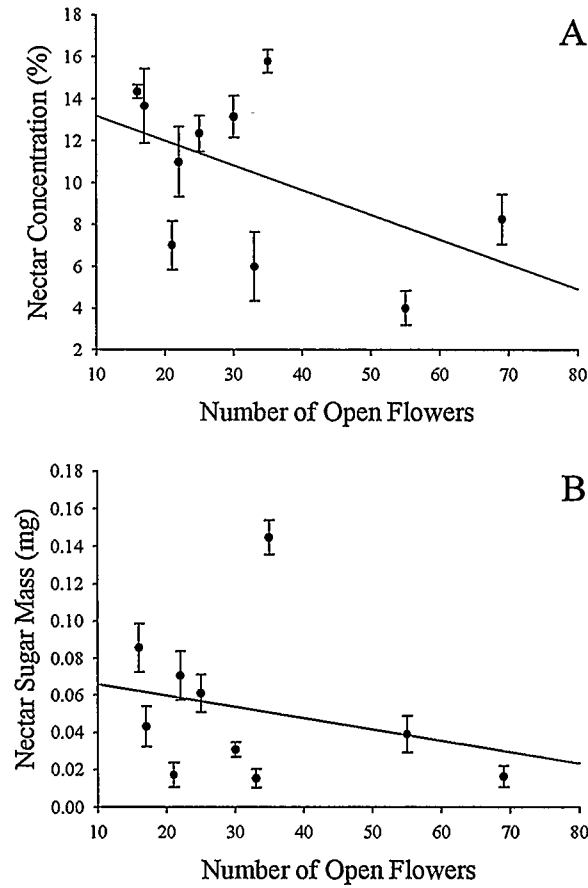


Figure 4.2: Relation of a) mean nectar concentration and b) mean nectar sugar production per flower to display size in *Platanthera dilatata* inflorescences for 10 plants from the Bostock population.

### 4.3.2 Pollinarium Removal

Pollinarium removal varied significantly among plants with different proportions of empty flowers ( $G_3=34.84$ ,  $P<0.001$ ; Figure 4.3a), among populations ( $G_7=13.82$ ,  $P<0.05$ ; Figure 4.3b), and with display size ( $G_1=18.53$ ,  $P<0.001$ ; Figure 4.3c) and the total number of pollinia on an inflorescence ( $G_1=14.26$ ,  $P<0.001$ ; Figure 4.3d). The number of pollinaria removed varied in an unexpected manner with the proportion of empty flowers on an inflorescence (Figure 4.3), as plants with many

empty flowers (66% and 100%) had twice the proportion of pollinaria removed (mean  $\pm$ SE =  $22 \pm 0.026\%$ ) as those with few empty flowers (0% and 33%,  $11.5 \pm 0.017\%$  removal). Pollen removal did not differ significantly between either the 0% and 33% empty treatments ( $P > 0.1$ ) or the 66% and 100% empty treatments ( $P > 0.25$ ). This pattern was largely consistent among populations (interaction between population and proportion of empty flowers,  $G_{21}=23.42$ ,  $P > 0.25$ ), as removal was higher in treatments with few empty flowers in only one population (Table 4.2). In general, the proportion of pollinaria removed from plants varied positively with the number of pollinaria (Figure 4.3d) and negatively with the number of open flowers (Figure 4.3c). The specific effect of display size is roughly consistent with pollinators removing about four pollinaria, regardless of the number of open flowers (Figure 4.3c).

Table 4.2: The average percentage of pollinaria removed from *Platanthera dilatata* inflorescences with different proportions of empty flowers for eight populations.

Population	% Empty Flowers			
	0%	33.33%	66.66%	100%
Bostock	17.7	16.2	32.8	27.7
Bostock Railway	22.2	3.7	18.2	15.4
Crowfoot Glacier	9.4	6.7	14.9	28.2
Glacier Hwy	11.1	9.1	30.9	32.1
Takkakaw Falls Meadow	7.1	19.8	25.9	19.2
Takkakaw Falls Roadside	13.2	15.5	10.7	9.2
Takkakaw Falls Stream	14.8	2.9	20.2	18.4
Yoho Hwy	14.2	8.2	10.5	30.4
<b>Average</b>	<b>13.7</b>	<b>10.3</b>	<b>20.5</b>	<b>22.6</b>

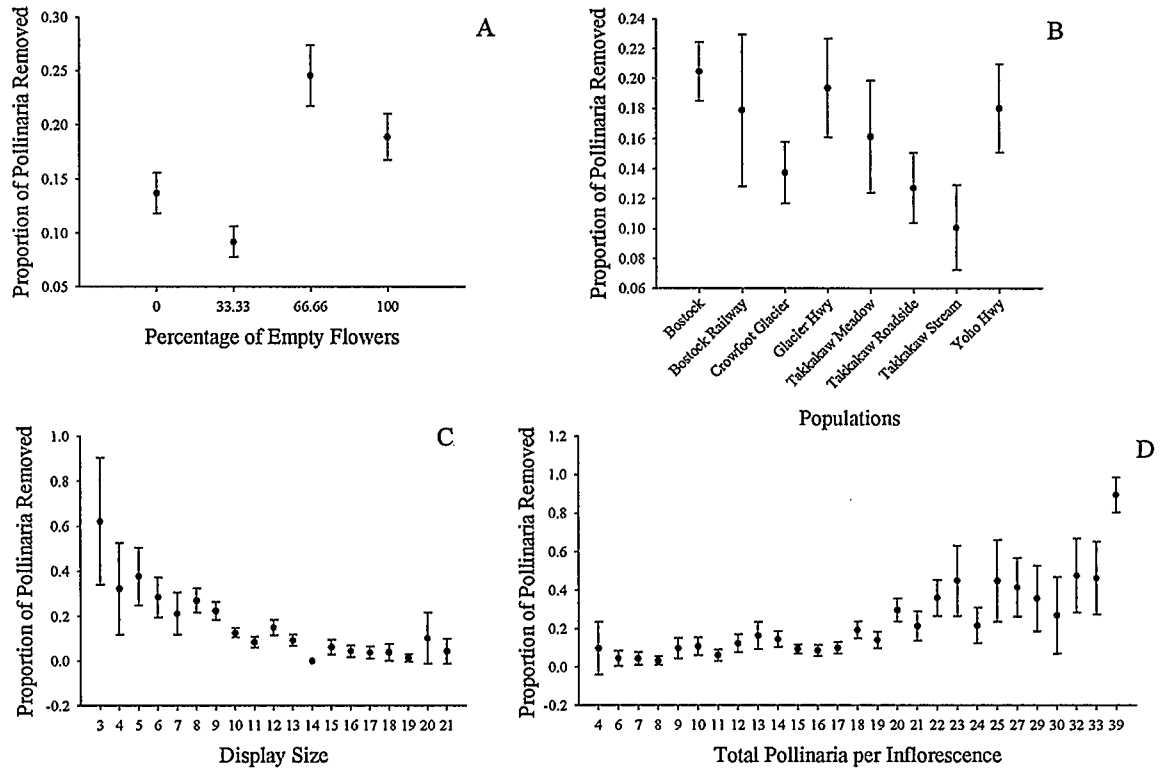


Figure 4.3: Significant influences on pollinarium removal from *Platanthera dilatata* inflorescences, including a) the percentage of empty flowers, b) population, c) inflorescence display size, and d) the total number of available pollinaria.

#### 4.3.3 Incidence of Self-pollination

Overall, 45% of the 98 plants on which I stained pollinia experienced some self-pollination during a single night's exposure to pollinators. The incidence of self-pollination varied negatively with the percentage of empty flowers ( $G_3=12.77$ ,  $P<0.005$ ), ranging from 36% of plants with no empty flowers to 9% of plants with all empty flowers (Figure 4.4). No other measured variable affected the incidence of self-pollination ( $P>0.05$  in all cases). Of the 44 plants that experienced

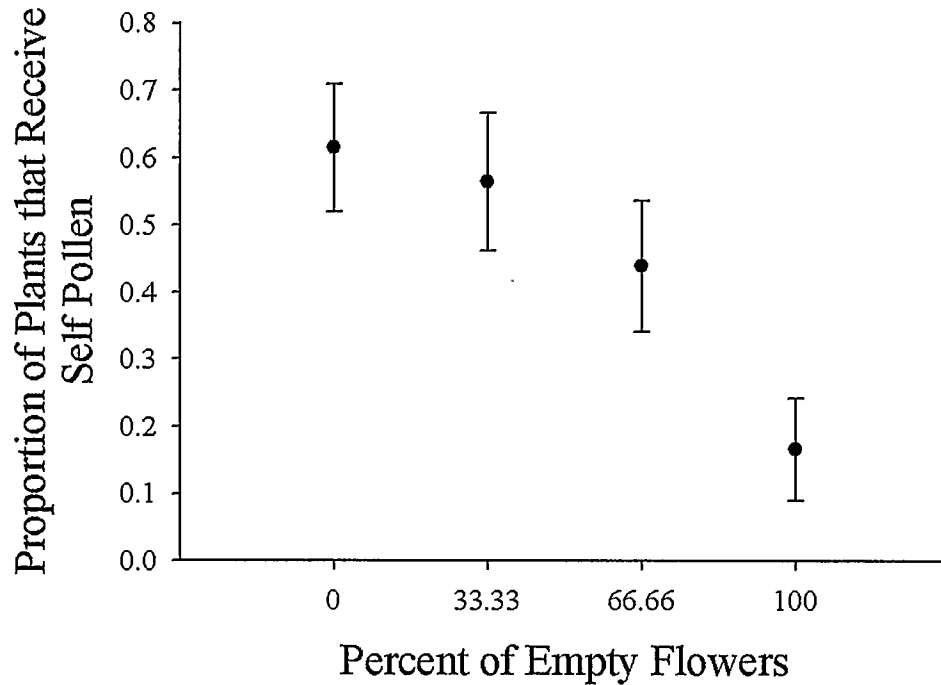


Figure 4.4: The effect of the percentage of empty flowers on the proportion of *Platanthera dilatata* inflorescences receiving at least one stained self-massula.

self-pollination only 16 (36%) had more than one flower self-pollinated and only two flowers of each of these plants received self-massulae.

#### 4.3.4 Pollen Export

Of the 98 plants on which I stained pollinaria, 46 (47%) exported massulae to the neighboring plants that I examined within 20 m. The number of flowers to which a plant exported pollen depended primarily on its proportion of empty flowers ( $G_3=27.38$ ,  $P<0.001$ ; Figure 4.5a), the population in which it lived ( $G_7=14.61$ ,  $P<0.001$ ; Figure 4.5b), the number of neighbouring plants sampled around a potential

pollen donor ( $G_1=16.29$ ,  $P<0.001$ ; Figure 4.5c), and a donor plant's display size ( $G_1=4.52$ ,  $P<0.05$ ; Figure 4.5d). However, the negative influence of display size resulted entirely from unusually high pollen export by the two plants with four flowers, as display size did not affect pollen export when these plants were excluded from the analysis. Plants with all empty flowers exported massulae to about three times more flowers than those with 0, 33 or 66% empty flowers, which did not differ from each other. The differences in the incidence of pollen export among populations resulted primarily from relatively high export in the smallest Bostock Railway population, which included only 24 plants, and relatively low export in the Glacier Highway population, which is located beside the busy Trans-Canada highway where winds created by passing vehicles frequently buffeted plants. Not surprising, the number of flowers to which a plant exported pollen varied positively with the number of potential recipient plants that I examined within 20 m of the stained plant (Figure 4.5c).

Overall, recipient plants lay within an mean ( $\pm$ SD) of 1.61 ( $\pm$ 1.25) m of donor plants and never farther than 5.5 m, even though I examined potential recipients within a 20-m radius. The mean distance between donor and recipient plants did not vary significantly with the percentage of empty flowers ( $F_{3,79}=1.37$ ,  $P>0.25$ ,  $R^2=0.05$ ).

## 4.4 Discussion

Overall, the proportion of empty flowers significantly influenced all measured aspects of the pollination of *Platanthera dilatata*. Specifically, plants with a high proportion of empty flowers had more pollinaria removed, a lower incidence of self-pollination and exported pollen to more flowers on other plants than those with relatively few empty flowers. I now discuss these findings separately.

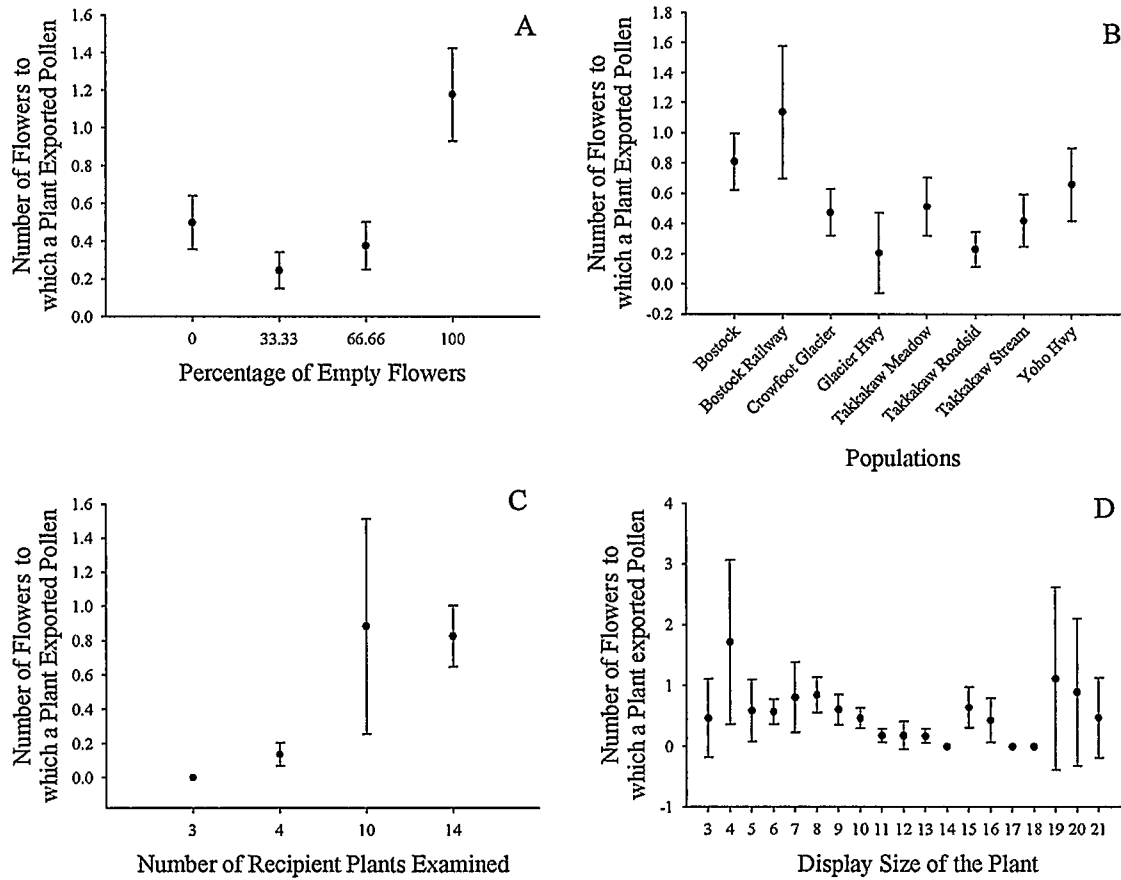


Figure 4.5: Factors that influenced the number of flowers to which *Platanthera dilatata* inflorescences exported massulae, including a) the percentage of empty flowers, b) the population to which a plant belonged, c) the number of potential recipient plants sampled around a focal plant, and d) the plant's display size.

#### 4.4.1 Pollen Removal

The elevated pollinarium removal for plants with many empty flowers contradicts the prediction that pollinators respond to limited rewards on an inflorescence by leaving a plant in search of more profitable plants. By leaving after encountering empty flowers, individual pollinators would each visit fewer flowers, and so remove fewer pollinaria. This contradiction between expectations and observations could have arisen from two causes; unintended effects of study design, or unexpected pollinator-plant interactions. I will consider aspects of study design before discussing possible pollinator responses that could account for my observations.

The observed effects of nectar manipulation do not reflect differing plant and population characteristics. Inflorescences that I manipulated to create different proportions of empty flowers did not differ significantly in display size ( $F_{3,94}=0.41$ ,  $P=0.75$ ), the number of available pollinaria ( $F_{3,94}=0.83$ ,  $P=0.48$ ), or plant density within a population ( $F_{3,94}=0.01$ ,  $P=0.99$ ). Moths probably did not detect variation in the absence of nectar on manipulated inflorescences before visiting flowers, because the elongated curved spur and its small opening conceal nectar at the tip of the spur.

Alternatively, the colour of the stains used to mark pollinia could have affected either the relative attractiveness of inflorescences subjected to different treatments, or removal of pollinia from their anther sacs, because I used the same stain for a particular treatment in all populations. However, Peakall (1989) found that staining did not affect the number of pollinia removed by pollinators in another orchid species.

Enhanced pollen removal from plants with many empty flowers is consistent with the proposal by Smithson and Gigord (2001) that pollinators continue to search for nectar in a flower after the initial proboscis extension yielded no rewards if they have previously experienced rewarding plants. Pollinators seem to be relatively uncommon

in the populations that I studied, as moths removed an average ( $\pm$ SD) of 2.85 ( $\pm$ 2.32) pollinaria per night from inflorescences with an average of 16.64 ( $\pm$ 6.58) pollinaria. Given low pollinator abundance, moths should seldom encounter empty flowers and I found that only 10 ( $\pm$ 3)% of flowers lacked nectar naturally. Consequently, moths encountering empty flowers in such a situation may probe an empty flower multiple times to verify that the flower lacks nectar, as I observed on several occasions. Such repeated probing should increase the probability of removing a pollinarium ( $p$ ), although this effect will tend to be counteracted by a reduction in the number of flowers that a moth visits on the inflorescence ( $f$ ) once detecting that it offers little reward. Together, these responses determine the proportion of pollinia removed ( $R$ ),

$$R = fp, \quad (4.1)$$

which could increase in response to empty flowers if the positive effect on the probability of pollinaria removal exceeds the decrease in the number of flowers visited per pollinator.

This hypothesis is supported by the contrasting effects of empty flowers on pollinarium removal and self-pollination, with inflorescences with a high proportion of empty flowers (66 and 100%) experiencing much less selfing than those with fewer empty flowers (0 and 33%; Figure 4.3a). Such reduced self-pollination in response to empty flower is consistent with moths visiting fewer flowers on manipulated inflorescences, leading to less geitonogamy. This diminished self-pollination would be assisted by the bending of the *Platanthera dilatata* pollinarium (see Boland, 1993). Once a pollinarium is removed from a *P. dilatata* flower the caudicle dries, so that the pollinium rotates into a forward position in which it can contact stigmas. As a result, a pollinarium cannot contribute to self-pollination within a minute of being removed



from its anther sac. This mechanism should both eliminate increased self-pollination within flowers caused by multiple probing and limit geitonogamy, if moths visit few flowers.

#### 4.4.2 Self-Pollination

Just under half of the *Platanthera dilatata* inflorescences on which I stained pollinia experienced self-pollination. The incidence of self-pollination decreased with the proportion of empty flowers, suggesting that empty flowers would help reduce self-pollination and its associated negative consequences (i.e. inbreeding depression: Barrett et al., 1994). This outcome supports the hypothesis that empty flowers enhance pollination. Moths visiting *P. dilatata* apparently left plants quickly after encountering a high proportion of empty flowers, thereby reducing the incidence of self-pollination.

As mentioned in the discussion of pollen removal, a *P. dilatata* pollinarium bends to allow massulae deposition on stigmas after about a minute, so that most self-pollination probably involves time-consuming pollen movement between flowers, rather than within flowers. Unlike many other plants subject to such geitonogamy, in which self-pollination varies positively with the number of open flowers (reviewed by Harder et al., 2004), display size did not significantly affect the incidence of self-pollination for *P. dilatata*. This result is consistent with my limited observations that moths visiting *P. dilatata* inflorescence typically probe 3-4 flowers per plant, regardless of display size. Apparently, an elevated proportion of empty flowers reduced this number, thereby diminishing geitonogamy.

The non-linear effect of the proportion of empty flowers (Figure 4.4) on the incidence of self-pollination may reflect moths' experiences with the natural frequency

of empty flowers. Nectar sampling in the Bostock population found 0 to 30% empty flowers per inflorescence, with an average of 10%. Consequently, pollinators encountering a proportion of empty flowers in the natural range would likely not be “convinced” to seek another plant, resulting in the equivalent incidence of self-pollination for my 0 and 33% empty treatment. In contrast, the 66 and 100% empty flowers greatly exceed the natural frequency of empty flowers and so are more likely to induce pollinator departure from inflorescences, reducing geitonogamy.

#### 4.4.3 Pollen Export

The number of flowers to which stained plants exported massulae also exhibited a nonlinear relation to the proportion of empty flowers, with a strong effect only when no flowers contained nectar (Figure 4.5a). Because I used the same stain for all plants subjected to the same treatment, this result could be an artifact of the staining procedure. Pollinia for the 100% empty treatment were stained with Bismarck brown. Unstained massulae also turn brown with age; however, their colour is appreciably darker than that of massulae stained with Bismarck brown, so it is unlikely that I mistakenly identified old unstained massulae for those stained with Bismarck brown. If misidentification did occur, it should also have affected my assessment of self-pollination for the 100% empty treatment, but this treatment experienced the lowest incidence of self-pollination. Furthermore, if aged pollinia were misidentified, I should have observed them throughout the population, but brown massulae were generally found on stigmas closer to the 100% treatment plants than the distance dispersed by massulae stained with the other colours. This result could occur if massulae stained with Bismarck brown disperse differently than those treated with other stains; however, previous studies have found no evidence of stain type affecting

massulae dispersal (Peakall, 1989; Johnson et al., 2005). Thus the elevated dispersal of pollen from completely nectarless plants seems to reflect the effect of empty flowers on pollen export, rather than a peculiarity of study design.

In the absence of treatment artifacts, my results indicate that a high proportion of empty flowers are required to affect pollen dispersal in *P. dilatata*; however, it is unclear why this trend does not increase with the proportion of empty flowers, as expected (Figure 4.5a). If a pollinator's threshold is between 66 and 100% empty flowers, then pollinators would respond to treatments less than or equal to 66% as was seen. In contrast, pollinium transport loss could explain reduced pollen export in the 0, 33 and 66% empty flowers treatment. Plants with a lower proportion of empty flowers offer better nectar rewards than those with a higher proportion, and pollinators may visit more flowers and spend longer per flower (Chapter 3), increasing pollen removal (Harder, 1990). Increases in pollinarium removal may increase pollinarium transport loss, as Johnson et al. (2005) suggested that a build up (clumping) of pollinia on a moth's proboscis increased the chance of the entire group of pollinaria falling off. Furthermore, a build up of pollinaria on a pollinator's proboscis could inhibit access of pollinia obtained previously to stigmas. Plants with a low proportion of empty flowers (particularly below a pollinator's threshold) would certainly be more susceptible to pollen transport loss than those with a higher proportion of empty flowers.

In conclusion, this experiment demonstrated diverse influences of empty flowers on pollination. An increased proportion of empty flowers increased pollinarium removal and pollen export, while decreasing self-pollination. These effects should ultimately enhance paternal reproductive success and perhaps maternal success, if *Platanthera dilatata* experiences inbreeding depression. Curiously, the greatest benefits to reproduction resulted from the highest proportions of empty flowers (66

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and 100%), even though *P. dilatata* plants naturally have an average of 10% empty flowers. However, it is important to bear in mind that my experimental manipulations occurred in the context of many neighbouring plants with low frequencies of empty flowers, so that the benefits of empty flowers may be frequency dependent. Other factors that determine the optimal proportion of empty flowers are discussed in Chapter 5.

## Chapter 5

# The Ecological and Evolutionary Significance of Empty Flowers

### 5.1 Functionally Empty Flowers

This thesis has considered the incidence and possible functions of nectarless flowers; however, I have not defined empty flowers explicitly. Most references to empty flowers describe them logically as having no nectar (Bell, 1986; Thakar et al., 2003). I similarly judged a flower to be empty if it contained no nectar; however, this assessment is short-term, as it was based on 24 h, rather than a flower's entire life. My analysis of pollinator behaviour on *Chamerion angustifolium* revealed that in populations with abundant pollinators most, if not all, flowers have very little nectar, so that frequent visitation renders flowers functionally empty for much of their lives. An intrinsic lack of nectar production can both save energy and alter pollinator behaviour, whereas extrinsic nectar depletion can serve only the latter function. Nevertheless, if flowers are typically "empty" because of recent visits, plants do not have to produce a high proportion of empty flowers to affect pollination. Under

these conditions, diminishing returns associated with pollen removal are minimal and pollen export would be maximal (Harder and Thomson, 1989). This combination of causes of empty flower may contribute to the low proportion of intrinsically empty flowers seen in many species.

Assessment of the influence of nectarless flowers as a pollination-enhancement strategy should ideally consider the effective number of both intrinsically and extrinsically empty flowers. Obviously, this effective number depends on the pollination environment, specifically on pollinator abundance within a population. Interestingly, the frequency of beneficial empty flowers increases with pollinator abundance, creating a situation in which pollinators would typically visit only a few flowers on a plant when restricted pollen removal is most beneficial for counteracting the effects of the diminishing returns associated with pollen export (Harder and Thomson, 1989) and self-pollination (Harder and Aizen, 2004).

This relation between the effective proportion of empty flowers and pollinator abundance suggests that a species' flowering circumstances influence the evolution of intrinsically empty flowers. Plants that do not compete for pollinators with other simultaneously flowering species may not require intrinsically empty flowers, if abundant pollinators create many functionally empty flowers.

## 5.2 The Optimal Proportion of Empty Flowers

My experimental analyses of the effect of nectarless flowers on pollinator behaviour (Chapter 3) and pollen transfer (Chapter 4) both suggest that high proportions of empty flowers (>50%) are beneficial. However, this conclusion contradicts the survey of 52 species, which found an average percentage of intrinsically empty flowers of 11 ( $\pm 2$ )% (Chapter 2). Why do natural populations show relatively low percentages of

empty flowers?

In addition to the proposal outlined above, that many flowers in many species may be empty because of frequent pollinator visits, plants may produce relatively few empty flowers as a reproductive bet-hedging strategy that reduces the consequences of unpredictable periods of low pollinator abundance. Seed production varies extensively in space and time, presumably because of variation in pollinator abundance (Goodwillie, 2001; Ashman et al., 2004; Herrera, 2005). When pollinators are uncommon, plants with no empty flowers would be at an advantage, as pollinators would visit multiple flowers and remain longer on each flower, maximizing pollen removal even at the expense of increased pollen loss and self-pollination (see Harder et al., 2001). With this bet-hedging strategy, it is better to self-fertilize and incur the cost of inbreeding depression and diminishing returns, than to suffer pollen removal failure and limited pollen receipt. Conversely, when pollinators are moderately abundant empty flowers would be advantageous for enhancing out-crossing, as described in Chapter 5. Thus, under variable conditions plants may adopt a low percentage of empty flowers (i.e. bet-hedge) as the best strategy for dealing with uncertain pollinator abundance. Indeed, species with a low proportion of empty flowers (as observed in the survey) can best tolerate the widest range of pollinator abundance that would likely be encountered within and among seasons. Species within this range have reproductive assurance during times when pollinators are scarce and provide enhanced pollen export when pollinators are abundant.

The optimal production of empty flowers may involve the ability of flowers to turn nectar production on and off during different periods of their lives, so that they may not be consistently nectarless. Such temporal variation in nectar production may be beneficial for species visited by pollinators that move predictably among flowers within inflorescences (i.e. upward foraging on racemes, or outer to inner flowers on umbels:

Jordan and Harder, 2006), if it allows precise placement of empty flowers within the inflorescence. A flower's position within an inflorescence is relative and changes as other flowers open and older flowers senesce. If empty flowers serve pollination more effectively in some positions, rather than others, then the ability of a plant to restart nectar production or inhibit nectar production in coordination with the maturity of its inflorescence would be greatly beneficial.

Previous studies of empty flowers have mostly involved mathematical modelling to examine the selection of nectarless flowers to promote energy-savings (Bell, 1986; Gilbert et al., 1991; Sakai, 1993) and enhance pollination (Smithson and Gigord, 2003; Thakar et al., 2003). This thesis is the first examination of both suggested strategies in natural populations, as well the effects of empty flowers on pollinator behaviour and pollen transfer in otherwise nectar-producing species. Although this thesis provides the framework to begin understanding the incidence of empty flowers and their function, much additional work is required.



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