

UNIVERSITY OF CALGARY

Assortative mating and local phenotypic selection in Platanthera dilatata (Pursh) Lindl.  
(Orchidaceae)

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES  
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE  
DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF BIOLOGICAL SCIENCES

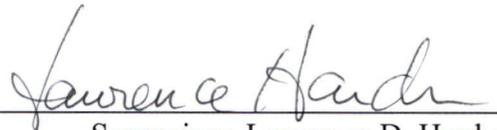
CALGARY, ALBERTA

SEPTEMBER, 2007

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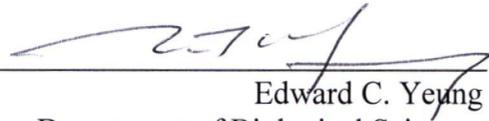
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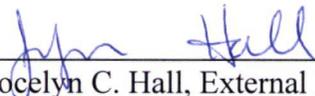
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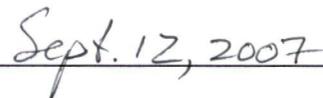
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## Abstract

This thesis considers the incidence of positive assortative mating mediated by floral morphology and spatial variation in phenotypic selection on floral traits in *Platanthera dilatata*. Although commonly cited as a mechanism promoting floral diversification, positive assortative mating has not been demonstrated for plants with monomorphic flowers. Observations from four populations revealed positive assortative mating by column length among flowers, but not among plants, because of extensive within-plant variation in this trait. Mating occurred randomly with respect to nectar-spur length, probably due to variation in spur curvature. The four study populations experienced significant phenotypic selection on column and spur length, as well as plant height, which generally favored larger plants with larger flowers. However, I found no evidence to contrasting selection among populations, presumably reflecting their close spatial proximity and similar selective environments. These results are discussed in reference to their evolutionary implications for flowering plants.

## **Acknowledgements**

My time at the University of Calgary has brought me in contact with some very brilliant and helpful people. Almost everyone I met has contributed to my positive experience. In particular I would like to thank several individuals who have not only contributed their suggestions and insights, but have been valuable mentors to me.

First and foremost, I would like to thank my supervisor Lawrence Harder. He has been very patient and supportive. His clear grasp of knowledge, precision in thinking and deep experience made being one of his students a privilege. I have been most fortunate to learn from such an excellent supervisor.

I would also like to thank members of my supervisory committee, John Addicott and Ed Yeung. Not only have they provided very valuable comments and technical assistance, but they were also great mentors, challenging me to think beyond the project that I was working on and to consider the implications of alternate perspectives. Another notable mentor is Steve Vamosi, with whom I had many inspiring conversations.

I enjoyed conversations with my fellow graduate students, in particular Tanya Latty, Ken Jefferies, Jeffery Tindall, Nina Hobbhahn and Robert Laird, who have often raised perspectives that challenged me to address my work in a more comprehensive way. Additionally they have made graduate school an immensely social experience. In particular I thank Nina Hobbhahn for her love and inspiration, without her the task of completing my thesis would have been far more arduous and psychologically bruising. Finally I thank my family for their unwavering support.

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## Chapter One: Introduction

Among the microevolutionary processes that generate biological diversity, natural selection is unique, because it alone produces adaptations that promote performance in specific environments. If populations differ in their relations of fitness to trait variation and/or the extent and nature of genetic variation and covariation, then they could follow different evolutionary trajectories, resulting in phenotypic divergence and ultimately speciation (Schluter, 2000). Consequently, factors that modify either the fitness relations within and among populations, or the genetic responses of populations to selection can influence the evolutionary outcome of local adaptation (Schluter, 2000). Nevertheless, the extent to which selection varies among populations is poorly understood (Kwiatkowski and Sullivan, 2002; McKay et al., 2005; Herrera et al., 2006). Differences in selection among populations must be caused by differences in the environmental conditions that determine the relations of fitness to traits and/or the genetic structure of traits. Short of being able to identify potential causes of selection variation, distances between populations can serve as a rough correlate, because geographically distant populations are more likely than adjacent populations to experience contrasting environments (Gilbert et al., 1996; Campbell et al., 1997; O'Connell and Johnston, 1998; Caruso, 2000; Gomez and Zamora, 2000; Irwin, 2000; Aspi et al., 2003). Herrera et al. (2006) reviewed studies of phenotypic selection on floral traits and found that 26 of 40 studies considered multiple populations. These studies commonly found variation in selection at the spatial scale of 4 km - 150 km. However, only 7 of the 40 studies they reviewed have found spatial variation in selection at the scale <4 km. Similarly, McKay

et al. (2005) reported that local adaptation is commonly observed at large spatial scales, but there is little evidence for smaller-scale local adaptation.

The direction and strength of selection can be influenced by mating patterns, because non-random mating creates associations among genes (Lande and Arnold, 1983; Armbruster and Schwaegerle, 1996; Schluter, 2000; Armbruster, 2002; Begin and Roff, 2003; Hansen et al., 2003; Caruso, 2004; Jones et al., 2004). Such genetic associations may influence the direction of selection, because genetic covariation constrains the ability of a gene to respond freely and independently to selection (Kirkpatrick and Lofsvold, 1992; Schluter, 2000). As a result, evolution will tend to follow the trajectory that is least genetically constrained, which may not lead to the most suitable adaptation in a given environment (Schluter, 2000; Begin and Roff, 2003; Jones et al., 2004). Non-random associations among genes can also influence the strength of natural selection, because genetic correlations allow direct selection on one trait to cause indirect selection on others (Lande and Arnold, 1983; Armbruster and Schwaegerle, 1996; Armbruster, 2002; Hansen et al., 2003). If the direction of direct selection is congruent with the trait association, then selection is strengthened, resulting in correlational evolution. In contrast, a trait association that is incongruent with the direction of selection weakens, or may even reverse, selection (Lande and Arnold, 1983; Armbruster, 2002).

Positive assortative mating is a particular form of non-random mating that occurs when individuals with similar phenotypes mate. Theoretically, this mating pattern can influence selection on mating characteristics through two consequences for the mode of selection. Under positive assortative mating, genes controlling different mating characteristics tend to become associated, because both parents contribute genes for

similar phenotypes. As described above, the resulting positive genetic correlations could bias directional selection, thereby influencing the evolutionary trajectory followed by a specific population. Consequently, different patterns of positive assortative mating can arise among populations with different distributions of a mating character, which could facilitate contrasting responses to directional selection, resulting in diversification. However, directional selection will tend to be opposed by stabilizing selection caused by assortative mating, because individuals with rare, extreme mating traits have a lower chance of mating with suitable partners than those with more common, average traits (Kirkpatrick and Nuismer, 2004).

Positive assortative mating for morphological and behavioral traits has been well studied in animals (Emelianov et al., 2001; Jiggins et al., 2001; Schliewen et al., 2001; McKinnon et al., 2004), but not in plants. This mating pattern has been established convincingly for plants only in studies of phenology (Weis and Kossler, 2004) and polyploidy (Husband and Schemske, 2000). In these examples, plants were more likely to mate with individuals with similar flowering times or gametic compatibility. Polyploidy, in particular, can be a highly resistant reproductive barrier, such that speciation can occur almost instantaneously (Husband and Schemske, 2000; Kennedy et al., 2006). Flower color could also be involved in positive assortative mating, if pollinators prefer the common color in a population (Waser and Price, 1983; Stanton et al., 1989; Kulkarni, 1999; Jones and Reithel, 2001). Plants may also mate assortatively with respect to height when pollinators move preferentially among individuals of similar stature (Waddington, 1979). Whether plants mate assortatively within species with respect to floral morphology remains largely unstudied. Floral morphology in species

with monomorphic flowers has been proposed to promote precise mating (but see Wilson, 1995), and such mating precision has been cited as a key mechanism in adaptive radiation of *Aquilegia* (Hodges and Arnold, 1994; Hodges, 1997) or convergent evolution of *Penstemon* (Castellanos et al., 2003; Castellanos et al., 2004). A few studies have demonstrated that floral traits can govern precise placement of pollen on pollinators' bodies (Maad and Nilsson, 2004) and manipulation of the length of nectar spurs is known to reduce reproduction (Nilsson, 1988; Johnson and Steiner, 1997). However, to the best of my knowledge, no study has demonstrated positive assortative mating among monomorphic plants by floral traits.

Floral morphology could promote positive assortative mating through two non-exclusive mechanisms. Pollinators could visit plants with similar floral morphology preferentially, so that assortment results from pollinator behavior. Traits such as flower size (Blarer et al., 2002) and the number of flowers displayed simultaneously (floral display size: Ohashi and Yahara, 2001) can influence pollinator preference. Alternatively, pollinators could visit plants indiscriminately, but pollen dispersal could occur most readily between plants with sexual organs of similar length, causing pollen exchange with similar locations on a pollinator's body. The opportunity for assortative mating by the latter mechanism depends on the precision of pollen placement, being more likely when a plant's anthers and stigmas contact the same location on pollinators consistently and pollen is not redistributed on pollinators' bodies during transport. Such precision is likely common in plants with the following floral morphology: bilaterally symmetric, tubular flowers (including species with nectar spurs), which control the position and orientation of pollinators during visits, and few anthers and a single stigma,

which contact a well-defined location on the pollinator (Eckert and Barrett, 1994b; Hodges, 1997; Sargent, 2004).

## **1.1 Objectives**

This thesis assesses the incidence of positive assortative mating for floral morphology, its consequences for phenotypic selection within populations, and the consistency of phenotypic selection among populations. After describing methods that I use repeatedly, I describe my assessment of assortative mating for a monomorphic species with bilaterally symmetric flowers with elongate nectar spurs. I then test the hypothesis that assortative mating among plants causes stabilizing selection on the mate-choice trait. Finally, I characterize variation in phenotypic selection among spatially close populations to examine the occurrence of fine-scale local adaptation.

## Chapter Two: General methods

### 2.1 Introduction

The occurrence, mode and strength of selection in nature has received considerable attention to understand its role in micro-evolutionary processes such as local adaptation (Brodie et al., 1995). The advent of the first practical statistical tool for detecting and quantifying phenotypic selection in the field (Lande and Arnold, 1983) along with more refined and specific techniques (Koenig et al., 1991; Brodie et al., 1995; Kingsolver et al., 2001; Hereford et al., 2004; Hamon, 2005; Stinchcombe, 2005) prompted widespread efforts to measure the occurrence and nature of phenotypic selection (Kingsolver et al., 2001; Hereford et al., 2004; Herrera et al., 2006). Much of the effort to estimate selection has focused on relating evidence of selection to some causal mechanism, through experiments or correlational studies.

Selection can affect a trait's population mean, variance and covariance with other traits, either independently or simultaneously (Endler, 1986; Brodie et al., 1995). Directional selection occurs when the trait mean either increases or decreases because of higher fitness of individuals that are larger or smaller than average, respectively. Stabilizing and disruptive selection both affect trait variance: stabilizing selection decreases variance because extreme individuals contribute few genes to subsequent generations; whereas disruptive selection increases variance because extreme individuals realize high relative fitness. Correlated selection occurs when selection on one trait acts indirectly on other traits with which it is genetically correlated.

Lande and Arnold (1983) devised the first practical statistical framework for estimating phenotypic selection in nature using multiple regression of a fitness

component on phenotypic traits. In such a model, partial regression coefficients that differ significantly from zero provide evidence for phenotypic selection on the associated trait (Brodie et al., 1995). Models that consider only the original trait measurement can be used to assess the occurrence and intensity of negative or positive directional selection, depending on the sign of the partial regression coefficients. Quadratic regression models that consider both the original trait measurement and the squared measurement additionally allow assessment of stabilizing or disruptive selection. In particular, negative partial regression coefficients for quadratic terms indicate stabilizing selection and positive coefficients indicate disruptive selection.

As with multiple regression in general (Kutner et al., 2005), the Lande and Arnold approach is subject to several limitations, including a trade-off between statistical power and the number of traits considered (Mitchell-Olds and Shaw, 1987), multicollinearity among independent variables and non-normality of residuals (Brodie et al., 1995). A model that considers few traits may not account for correlational selection (Lande and Arnold, 1983). On the other hand, a model that considers many traits is particularly susceptible to reduced statistical power. The trade-off between accounting for correlational selection and statistical power can be overcome only with extremely large datasets and/or careful selection of independent variables (Mitchell-Olds and Shaw, 1987). Inclusion of many independent variables and quadratic terms also increases the chance that they are correlated with each other, which can reduce the accuracy of hypothesis tests (Kutner et al., 2005). Such multicollinearity among traits can be reduced by eliminating independent variables that contain repetitive information and careful *a priori* selection of independent variables. Multicollinearity between linear and quadratic

terms can be eliminated by centering traits on zero by subtracting the mean from all observations. This approach is typically accompanied by division of the trait mean for an observation by the standard deviation (standardization). Standardization of both fitness (dependent variable) and traits (independent variables) facilitates comparison of results among studies (Lande and Arnold, 1983).

The regression approach of Lande and Arnold involves general linear models, which require normally distributed residuals to allow accurate hypothesis testing (Kutner et al., 2005). In contrast, non-normality of residuals is common in selection analyses, because many fitness measures involve counts and discrete data (such as number of offspring), which can be strongly skewed. An alternate approach involves the use of generalized linear models that allow for a broader variety of sampling distributions (McCullagh and Nelder, 1989), while retaining the linear form and the associated interpretations concerning the mode of selection. Unfortunately, the approach of standardizing fitness measures to allow direct comparison of selection gradients among models cannot be applied for generalized models of fitness measures with non-negative distributions (e.g., binomial, Poisson and negative binomial) because standardization will produce some observations below zero and these will be excluded in the analysis.

## **2.2 Methods**

As described in the following chapters, I used Lande and Arnold's (1983) approach to estimate phenotypic selection for *Platanthera dilatata* (Pursh) Lindl., a terrestrial orchid, to examine both the association of positive assortative mating and stabilizing selection, and contrasting selection among populations. Each *P. dilatata* flower produces two pollinaria, which pollinators can remove separately, and each

pollinium is composed of smaller pollen units (massulae) joined with viscin, so that pollen from a single pollinium can be deposited on stigmas of multiple flowers. I studied phenotypic selection on the lengths of the sexual column (fused stamen and style) and nectar spur and in some cases I also considered selection on plant height. The following methods were applied in all cases.

### ***2.2.1 Stratified random sampling***

To assure that plants with uncommon (small or large) floral traits were sampled adequately, I used stratified random sampling to select study plants in each population. I characterized the frequency distribution of the combined column and spur length for 15-20 plants (depending on the number of available individuals) at fixed distances along the longest possible linear transect in 10-m patches of plants. With these measurements I constructed the cumulative frequency distribution of column+spur length and identified the four trait lengths that divided the distribution into five portions with equal numbers of plants (e.g. Figure 2.1). Within each patch, I then randomly chose one to three plants (depending on the number of available individuals) to represent each of the five portions of the cumulative frequency distribution. Stratified random sampling emphasizes the representation of the tails of the plant-trait distribution and facilitates sampling, because plants in each category have equal probabilities of being found when randomly choosing plants.

### ***2.2.2 Trait and fitness measures***

To facilitate comparisons of trait size among plants and account for variation in floral trait size due to flower age, I measured column and spur lengths on the oldest

functional flower on each plant with digital calipers (following Bateman and Rudall, 2006). I also measured plant height for all sampled plants from the ground to the top of the inflorescence. I measured four fitness components: the total number of flowers with pollinaria removal, the total number of massulae received by all stigmas, average number of fertilized ovules per flower and average seed production per flower. For pollen removal, I marked all flowers containing two pollinaria and counted the number of flowers from which at least one pollinarium was removed after 8-9 days (the observation duration). I estimated pollen receipt by marking all flowers with clean stigmas and counting the massulae received after 8-9 days with a 10x handlens. After flowers wilted and fruits matured, I divided each inflorescence into sections of five adjacent fruits and then randomly selected one capsule from each section, which I placed in an individually marked envelope until seeds could be counted.

To count seeds, I dissected each capsule in a small petri dish under a dissecting microscope (at 16x) and scraped out all the seeds and unfertilized ovules. I washed the seeds and ovules into a plastic vial with distilled water, adjusted the mixture volume to 20 mL and added 1 mL of 1% (v/v) Tween-20 (a surfactant) to break surface tension. To evacuate air from the testa, so that seeds and ovules would settle during counting, I loosely covered the vials and subjected them to a -90 kPa vacuum for 15 min. I then decanted the mixture carefully, reducing the volume to 10 mL, vortexed the sample and extracted two 1-mL subsamples. I counted all seeds in each subsample under a dissecting microscope (25x). Mature seeds were distinguished from ovules by the presence of an enlarged embryo.

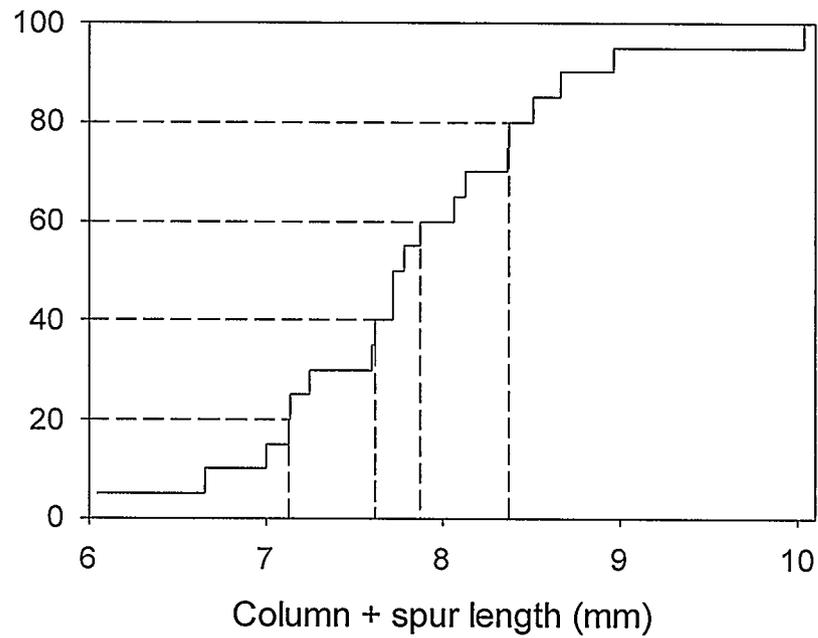
### *2.2.3 Analysis of phenotypic selection*

I estimated the form and strength of phenotypic selection on floral traits with quadratic regression based on Lande and Arnold (1983). The overall distributions of all four fitness measures were not normally distributed, but instead included many zero observations and a range of non-zero observations (see Figure 4.1). I mitigated this problem by conducting two analyses for each fitness measure. The first analysis considered the relative incidence of unsuccessful reproduction (zero observations) and successful reproduction (non-zero observations). This binary response was analyzed with a generalized linear model that considered a binomial distribution and used a logit link function to characterize the relation of mean fitness to plant traits (McCullagh and Nelder, 1989). The second analysis considered influences on only the normally-distributed, non-zero responses using general linear models (Figure 4.2) (Kutner et al., 2005). To interpret the two analyses of each independent variable, I consider significant selection gradients from the generalized linear models as evidence for selection on the probability of successful reproduction and significant selection gradients from the general linear models as evidence for selection on the magnitude of the fitness of reproductive individuals. All analyses also included terms for the number of available pollinaria, local neighborhood density and duration of observation to account for variation in plant size, local environment and sampling intensity, respectively. I estimated the linear and quadratic selection gradients in separate models (Brodie et al., 1995).

I standardized independent variables for all analyses and dependent variables for general linear models by subtracting the estimated population mean from each observation and dividing by the standard deviation. Because this transformation centers

the distribution of trait values on 0, it disrupts multicollinearity between standardized values and the squared standardized values used to test quadratic effects (Kutner et al., 2005). Additionally standardization of fitness components facilitates comparison of the estimated partial regression coefficients (phenotypic selection gradients) among traits and populations (Lande and Arnold, 1983).

To confirm the interpretation of significant selection gradients for metric fitness components, I plotted variation in fitness components against individual standardized traits after adjusting for the effects of other terms in the regression model. Adjusted predicted fitness was calculated for each observation with the estimated regression equation and the means for all traits, except the trait of interest. Adjusted observed fitness was then calculated by adding the residual from the overall regression model to an observation's adjusted predicted value.



**Figure 2.1** Sample cumulative frequency distribution of column + spur length from 20 plants in one patch in a *Platanthera dilatata* population.

Dotted lines denote cut-offs that divide the distribution into five parts with equal plant frequency.

## Chapter Three: Assortative mating in populations of *Platanthera dilatata* (Pursh)

### Lindl. (Orchidaceae)

#### 3.1 Introduction

Animal-pollinated angiosperms exhibit remarkable diversity in floral morphology. This diversity is typically interpreted as the product of selection favoring increased pollination efficiency and mating precision, which in turn promotes reproductive isolation (Nilsson, 1988; Eckert and Barrett, 1994b; Hodges, 1997; Barrett, 2002; Castellanos et al., 2003; Maad and Nilsson, 2004; Campbell and Aldridge, 2006; Johnson, 2006). Of particular relevance to this hypothesis are floral traits that govern the location and consistency of contact of reproductive organs with particular sites on pollinators (Bawa, 1995; Waser, 2001), especially the lengths of styles and stamens and the depth of nectar (Eckert and Barrett, 1994b; Hodges, 1997; Castellanos et al., 2003; Sargent, 2004). Heterostylous species provide the clearest example, as discrete, reciprocal differences in the positions of anthers and stigmas between morphs cause pollinators to carry pollen from each morph on different sites on their bodies, facilitating pollen transfer among sexual organs of similar lengths and thus disassortative mating between morphs (Barrett, 2002). In contrast, few experimental studies have shown that floral traits of species with monomorphic flowers enhance mating precision. Nevertheless, many studies cite precise mating governed by floral traits as a prime influence on floral evolution and diversification (Nilsson, 1988; Eckert and Barrett, 1994b; Hodges, 1997; Alexandersson and Johnson, 2002; Barrett, 2002; Sargent, 2004).

If flower depth and the lengths of reproductive organs control where on pollinators plants exchange pollen, monomorphic species with continuous variation in stamen and style lengths should mate assortatively (Cresswell, 1998), rather than randomly, with respect to floral morphology. Specifically, individuals should mate with other plants of similar stamen and style lengths, rather than with plants of dissimilar lengths. To the extent that trait differences among individuals are genetically controlled, such positive assortative mating could influence floral diversification in two ways. First, positive assortative mating causes stabilizing selection on traits that govern mating if reproduction is limited by mating opportunities (Kirkpatrick and Nuismer, 2004). This selection arises because individuals with the common morphology have more potential mates than those with less common morphologies. If populations differ in their frequency distributions for mating traits, the resulting dissimilarity in the details of stabilizing selection should sharpen morphological differences among them. Second, assortative mating creates gametic-phase disequilibrium among female and male mating traits within populations, which in turn creates genetic correlations that govern the course of evolution of these traits (Lande and Arnold, 1983; Armbruster and Schwaegerle, 1996; Schluter, 2000; Armbruster, 2002; Begin and Roff, 2003; Hansen et al., 2003; Caruso, 2004; Jones et al., 2004). Variation in genetic correlations among populations may lead to different responses to selection on mating traits (Kirkpatrick and Lofsvold, 1992; Schluter, 2000), promoting diversification.

Positive assortative mating should be most prevalent for species with anthers and stigmas that contact the same location on pollinators consistently and when pollen is not redistributed on the pollinator's body during transport. The first condition should apply

for species with bilaterally symmetric, or zygomorphic, tubular flowers (Sargent, 2004), including species with nectar spurs, because these floral traits constrain the position, orientation and movement of pollinators during visits (Bawa, 1995; Waser, 2001). Consistent contact with pollinators will be further enhanced if flowers have few anthers and a single stigma that contact a well-defined location on the pollinator (Eckert and Barrett, 1994b; Hodges, 1997; Sargent, 2004). The second condition should apply for species with pollinators that seldom groom, and/or that attach pollen firmly to pollinators' bodies.

Orchids possess all of the characteristics that should promote positive assortative mating with respect to floral traits. Their flowers are zygomorphic and often elongated into a nectar spur, which together impose stereotypic positions on nectar-feeding pollinators (Rudall and Bateman, 2002; Sargent, 2004; Gomez et al., 2006). Stamen number is much reduced in most orchids and the one or two stamens and style are fused into the column, which presents the anther(s) and stigma in close proximity (Dressler, 1993), so that a flower donates and receives pollen from the same location on a pollinator's body (Maad and Nilsson, 2004). Finally, most derived orchids produce aggregated pollen packages (pollinaria), which attach firmly to pollinators via a sticky viscidium, greatly reducing pollen losses during transport (Harder and Johnson, in press). Thus, orchids should commonly mate assortatively with respect to floral morphology, as long as floral traits vary more among individuals than among flowers on the same individual (Bateman and Rudall, 2006). Another potential determinant of mating precision is traits that govern the consistency of such interactions. Floral traits vary within individuals (Bateman and Rudall, 2006) and plants with less within-plant trait

variation should have more opportunity to mate assortatively, because of greater consistency of pollen exchange with a particular location on pollinators' bodies.

In this chapter I test the hypotheses that monomorphic spurred plants (specifically orchids) mate assortatively with respect to floral traits and that the resulting mating patterns lead to stabilizing selection on these traits. First, I quantify mating patterns among plants by tracking pollen exchange in four populations. Then, I assess the relative contributions of among- and within-plant variation in floral traits to total trait variation within populations to test whether the observed mating patterns could lead to evolutionary responses. Finally, I test whether assortment promotes stabilizing selection on mating traits by estimating phenotypic selection on the floral traits responsible for the existing mating patterns.

## **3.2 Materials and methods**

### **3.2.1 Field methodology**

I studied mating patterns and phenotypic selection of *Platanthera dilatata* (Pursh) Lindl., a terrestrial orchid pollinated primarily by noctuid moths that visit flowers to extract nectar from the elongate nectar spur (Boland, 1993). This species produces large spikes of ~80 white, scented flowers (Figure 3.1). Each flower produces two pollinaria, which can be removed separately, and each pollinium is composed of smaller pollen units (massulae) joined with viscin, so pollen from each pollinium can be deposited on stigmas of multiple flowers. I chose *P. dilatata* as the study species because its elongate nectar spurs and fused reproductive organs provide suitable conditions for assortment and its sectile pollinarium can be stained and tracked among plants within populations, mating patterns can be quantified directly.

I studied four populations of *P. dilatata* in Banff and Glacier National Parks of the Canadian Rockies (see Table 3.1). All populations occupy boggy sites within 20 m of seasonally water-logged roadside ditches. These populations were selected because they each contained more than 200 plants and were large enough to be sub-divided into at least three patches each with a 10-m radius. Populations sheltered by trees from adjacent road disturbance and with active pollinators were also favored for experimentation.

To assess mating performance I measured pollinarium removal and pollen export as male measures and massula receipt as a female measure. To quantify pollen export I stained all available pollinia in flowers of focal donor plants and later examined the stigmas of all neighboring plants within ~10 m for stained massulae (Peakall, 1989). Each study area was divided into patches with a radius of 10 m and five donors were selected within the center of each patch. A different colored histo-chemical stain (0.5% w/v amaranth acid red, 0.1% w/v orange g, 0.5% w/v fast green, 0.5% w/v methyl blue, 0.2% v/v gentian violet) was used for each donor, so that their pollen could be identified uniquely on stigmas. For every donor plant I measured the following traits for the oldest (lowermost) non-wilted flower: column length, spur length, maximum floral width, and maximum labellum length. I also measured plant height, inflorescence length and the density of plants within 0.33 m<sup>2</sup> centered on the focal plant.

To assure that plants with uncommon (small or large) floral traits were sampled adequately I used stratified random sampling (Chapter 2) to select potential pollen donors for the mating study in each population. Within each patch, I randomly chose five pollen donors with the condition that one donor represented each of the five portions of the cumulative frequency distribution.

Eight or nine days after pollinium staining I examined the stigmas of every *P. dilatata* flower within a patch for stained massulae. For each flower that had received stained massulae (recipient flowers) I measured the same floral and plant traits that I had measured on donor plants and recorded the number and color of massulae deposited. To facilitate comparison with donor plants, I also measured the floral traits of each recipient plant's lowermost nonwilted flower. I also measured the distance separating each pair of recipient and donor plants. At this time I also recorded pollinium removal and/or deposition of unstained and stained massulae for each flower on donor plants and measured the traits of flowers that had experienced pollen removal and/or deposition.

Assortative mating is most likely if floral traits vary less within individuals than among individuals. To assess this condition I characterized the variation of column and spur lengths associated with flower age and position along the inflorescences of 20 randomly selected *P. dilatata* plants in two populations. For each plant, I divided the inflorescence into five equal sections and randomly selected one flower from each section for measurement. Each flower was measured every 2-3 days from the day when it became capable of pollen export until it wilted.

I tested whether assortative mating caused stabilizing selection by quantifying phenotypic selection on column and spur lengths. To assess the association between fitness and trait variation, I measured the column and spur lengths and four components of fitness for all sampled plants (see Chapter 2), including: number of flowers that had pollinia removed, unstained massula receipt, average number of fertilized ovules per flower and average seed production per flower.

### ***3.2.2 Data analysis***

#### **3.2.2.1 Mating patterns**

If plants mate assortatively, the floral traits of pollen recipients should vary positively with those of the associated pollen donors with a slope of 1. I tested this expectation with mixed-model regression analyses (SAS), that accounted for repeated measurement (i.e., multiple recipients) of pollen donors (Kutner et al., 2005). Two types of regressions were performed: flower-level regressions that considered the average traits of flowers on recipient plants that received stained pollen from a particular donor and the average traits of flowers on the donor plant that experienced pollinarium removal; and plant-level regressions that considered the traits of the lowermost nonwilted flowers for donor and recipient plants. These two types of analyses serve to disentangle among-plant mating patterns from the underlying process of among-flower mating. If floral traits vary little within a plant, both approaches would produce similar results. Conversely, if floral traits vary extensively within plants, flower-level regression should find stronger evidence of assortative mating than plant-level regression.

I used weighted means of floral traits for donors and recipients for the flower-level regression analysis to account for different involvement of specific flowers in pollination. For example, if two flowers on a donor plant experienced pollen removal, but both pollinaria were removed from one flower and only one from the second, the first flower's traits had twice the weight of the second flower in the calculation of a plant's mean floral traits. Similarly, the traits of flowers on a recipient plant were weighted in proportion to their receipt of pollen from a specific donor. I estimated the partial regression coefficients (slope) for donor traits with a mixed model that incorporated a

variance-component covariance matrix and tested them against the expected value of 1 with a single sample *t*-test.

#### 3.2.2.2 Within- and among-plant variation in floral traits

Assortative mating can have evolutionary consequences only if floral traits vary more among plants than within plants. To assess the relative sizes of within- and among-plant variance components, I used mixed-model ANOVAs with a compound symmetric variance-covariance structure. These analyses considered column length, nectar-spur length and their sum as dependent variables, flower age as a fixed categorical factor and population, plants nested within populations and flowers nested within plants as random factors. I estimated the variance components and their standard errors for all random factors using restricted maximum likelihood (Kutner et al., 2005).

I also used mixed-model ANOVA to determine whether column and spur lengths varied with flower age and position within inflorescences, both treated as fixed factors. Population was also included in these analyses as a fixed factor. This analysis incorporated a compound-symmetry model of variation and covariation among flower positions within individual plants.

#### 3.2.2.3 Phenotypic selection

I estimated the nature and strength of phenotypic selection on floral traits with quadratic regression based on Lande and Arnold (1983), as described in Chapter 2, for the population that accounted for 60% of the mating observations (Bow 2). This analysis specifically tested for stabilizing selection on standardized spur and column lengths, so both original and squared measures of these traits were included as independent

variables. In such analyses stabilizing selection is indicated by a partial regression coefficient  $<1$  (Lande and Arnold, 1983). These analyses also included plant height, local neighborhood density and the duration of exposure to pollinators as independent variables to account for variation in plant size, local environment and sampling intensity, respectively.

### 3.3 Results

#### 3.3.1 Mating patterns

I found stained massulae from 17 of the 116 stained plants on the stigmas of 20 recipient plants, with 12 observed mating events occurring in one population (Bow 2). These 20 mating events provided evidence of positive assortative mating among flowers for column length, but not for spur length, or the sum of column and spur length (Figure 3.2). In particular, flower-level regression of recipient column length on that of the donor revealed a positive association ( $b \pm SE = 0.733 \pm 0.317$ ,  $t_{18} = 2.32$ ,  $P < 0.05$ ; Figure 3.2A) that did not differ significantly from the expected value of 1 ( $t_{18} = 0.838$ ,  $P > 0.4$ ). In contrast, regressions of recipient traits on donor traits did not detect significant associations for either spur length ( $0.223 \pm 0.250$ ,  $t_{18} = 0.89$ ,  $P > 0.3$ ; Figure 3.2C), or the combined column and spur length ( $0.238 \pm 0.254$ ,  $t_{18} = 0.94$ ,  $P > 0.3$ ; Figure 3.2D). In contrast to the flower-level regression results, plant-level regressions detected only random mating (slope not significantly different than 0) among donors and recipients for column length ( $0.219 \pm 0.215$ ,  $t_{18} = 1.02$ ,  $P > 0.3$ ) (Figure 3.2B), spur length ( $0.092 \pm 0.223$ ,  $t_{18} = 0.41$ ,  $P > 0.6$ ) and column+spur length ( $0.069 \pm 0.222$ ,  $t_{18} = 0.31$ ,  $P > 0.7$ ).

Mating tended to involve plants growing at similar neighborhood density within 0.33 m<sup>2</sup> ( $b \pm SE = 0.394 \pm 0.146$ ,  $F_{1,15}=7.27$ ,  $P<0.05$ ; Figure 3.3). This association does not reflect close proximity of mates, because inter-plant distance did not influence this regression significantly ( $F_{1,14}<0.25$ ,  $P>0.6$ ). Only 3 out of 20 of the mating pairs were within the 0.33 m<sup>2</sup> area for the measurement of plant density.

### ***3.3.2 Within- and among-plant variation in floral traits***

Floral traits varied more among flowers within plants than among plants. Column length exhibited almost equal variation among levels (within plant variance component  $\pm SE = 0.027 \pm 0.0099$ ; among plants,  $0.022 \pm 0.011$ ), whereas spur length varied almost twice as much within plants ( $0.559 \pm 0.1306$ ) as among plants ( $0.287 \pm 0.1485$ ). Consequently, within-plant variation in combined column and spur length ( $0.828 \pm 0.1815$ ) was more than double that among plants ( $0.337 \pm 0.1866$ ).

Column and spur lengths varied significantly with both flower position (summed lengths,  $F_{4,171}=46.47$ ,  $P<0.001$ ; Figure 3.4A) and floral age ( $F_{3,172}=12.74$ ,  $P<0.001$ ; Figure 3.4B). Average flower size was relatively constant for flowers in the bottom 40% of inflorescences, but declined for higher flowers (Figure 3.4A). Overall, flowers grew after anthesis, although this increase occurred primarily between a flower's third and fourth days (Figure 3.4B).

### ***3.3.3 Phenotypic selection***

I observed significant disruptive phenotypic selection in the Bow 2 population, which contributed most of the mating observations, rather than the stabilizing selection expected from positive assortative mating (Table 3.2). Column length experienced

disruptive selection for the probabilities of both pollinarium removal and massula receipt (Table 3.2). Spur length was also subject to disruptive selection for these variables and additionally for the number of pollinaria removed from plants that experienced some removal (Table 3.2).

### **3.4 Discussion**

#### ***3.4.1 Mating patterns and the implications of within-plant floral variation***

At least three ecological mechanisms could result in positive assortative mating within plant populations: contrasting flowering times between individuals (Weis and Kossler, 2004); pollinator preferences for floral traits (see Waser and Price, 1983; Stanton et al., 1989; Kulkarni, 1999; Jones and Reithel, 2001), or plant height (Waddington, 1979); or the influence of floral traits on the location of pollen exchange with pollinators' bodies. This study presents the first empirical evidence for the latter mechanism, as dispersal of *Platanthera dilatata* pollen primarily occurred between flowers with similar column lengths (Figure 3.2A). Previous evidence consistent with positive assortment by floral traits involved either interspecific correlations between floral morphology and the incidence of hybrid mating (Hodges and Arnold, 1994; Hodges, 1997), or single-species experiments that assessed the effects of manipulation of floral traits beyond their natural variation on pollen removal and deposition and fruit set, but not on actual mating patterns (Nilsson, 1988; Johnson and Steiner, 1997). The observed mating patterns among flowers with respect to column length demonstrate the possibility of assortative mating for species that exhibit continuous variation in the lengths of reproductive organs. If floral traits do not vary significantly among plants, then the floral-level assortment should

translate into similar plant-level assortment. This result provides the first direct evidence for assortative mating caused by variation in the lengths of floral organs.

Despite evidence of positive assortative mating for column length among flowers, I found no such pattern among plants (compare Figure 3.2A with Figure 3.2B). This contrast may have resulted from limited statistical power, as I observed only 20 mating events. All 116 plants on which I stained pollinia experienced pollinarium removal (481 pollinaria removed in total), yet only 17 were found to have exchanged stained massulae with recipient plants within 10m. This low observed incidence of pollen export, combined with the long exposure to pollinators (8-9 days), indicates pollen dispersal beyond 10m and/or considerable pollen loss during transport in these populations. Similar incidence of pollen loss was found for *Disa cooperii*, another massulate orchid (Johnson et al., 2005). Alternatively, the extensive variation in floral morphology among flowers on individual plants (Figure 3.3) suggests a more likely biological explanation. In particular, within-plant variation may dilute the plant-level effect of assortative mating among flowers. For example, the smaller flowers (e.g., young flowers or those high on the inflorescence) on a plant with generally large flowers may exchange pollen with larger flowers (e.g., old flowers or those low on the inflorescence) on plants with generally small flowers. Indeed, within-plant variation that approaches or exceeds the extent of among-plant variation, as in *P. dilatata*, essentially makes almost every plant in a population a potential mate for the average plant, regardless of the precision of pollen exchange with pollinators. In this situation, assortative mating among floral phenotypes can have limited evolutionary consequences, because inter-plant mating occurs randomly with respect to floral genotypes. Given that trait size commonly varies among flowers

within plants (Bateman and Rudall, 2006), positive assortative mating at the plant level may typically be relatively weak.

The absence of positive assortative mating among flowers based on nectar-spur length (Figure 3.2C) is surprising, as nectar spurs have been identified as important floral structures that govern mating precision among plants (Nilsson, 1988; Barrett, 1990; Hodges, 1997; Johnson and Steiner, 1997; Schluter, 2000; Barrett, 2002; Castellanos et al., 2003; Sargent, 2004). Again, failure to observe assortative mating for spur length may reflect the impact of a small sample on statistical power. However, I observed extensive variation in the curvature of *P. dilatata* nectar spurs (Figure 3.1), which may limit assortative mating. The nectar spurs of most *P. dilatata* flowers curve strongly, so the distal portion of the spur is oriented at 5°-90° compared to the basal portion. I straightened nectar spurs before measuring them, whereas moths probing for nectar must contend with bent spurs. Moths may not need to probe the entire length of a bent spur to access its nectar, so variation among plants and flowers in spur curvature may induce variation in the location of pollen exchange, causing random mating with respect to spur length and thus for combined spur and column length.

I detected a significant association in the local neighborhood density of plants that mated with each other, which cannot be explained by limited interplant distances (Figure 3.3, compare symbols of different types). One possible explanation for density-based association is the idiosyncrasy of pollinator behavior.

### ***3.4.2 Phenotypic selection***

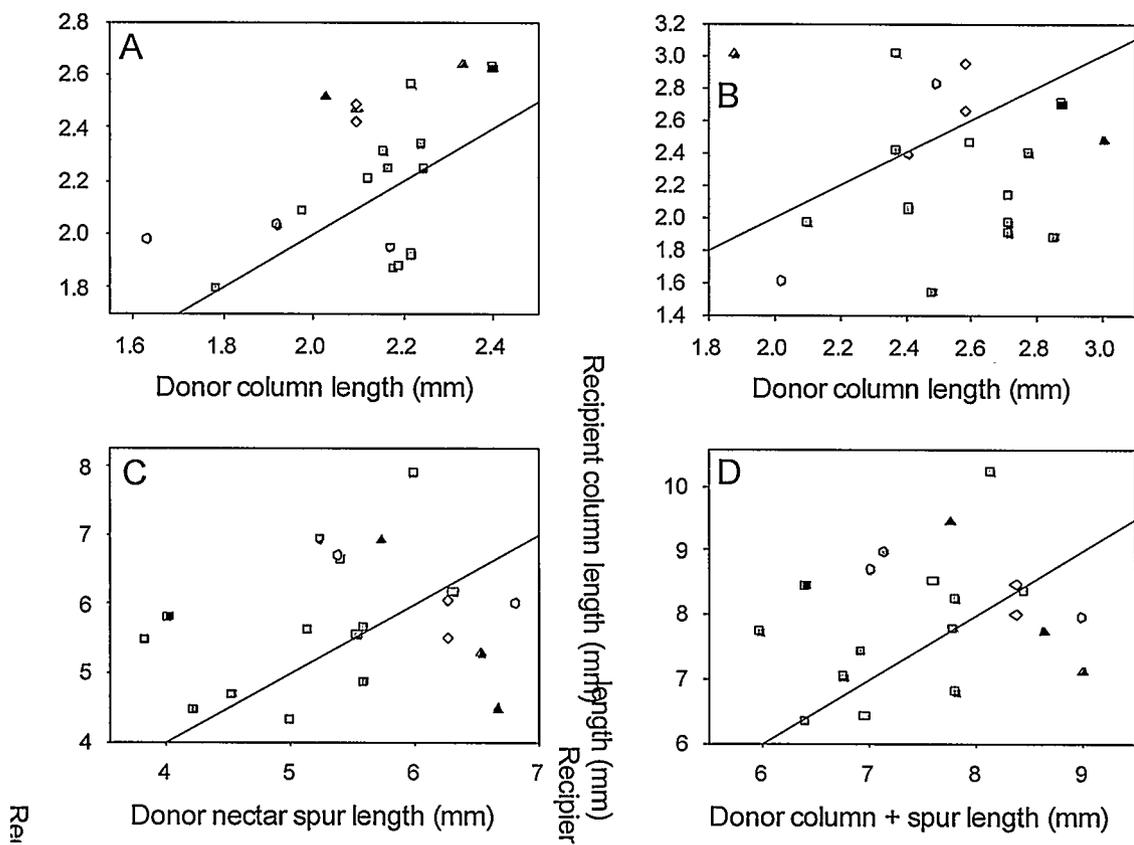
In contrast to the stabilizing selection that should accompany assortative mating (Kirkpatrick and Nuismer, 2004), I detected disruptive phenotypic selection on column

and spur lengths. The failure to observe stabilizing selection is not surprising, given that extensive within-plant variation in floral traits hinders consistent assortative mating among plants. Thus, the observed selection must have arisen from fitness differences caused by unknown processes, other than the unequal mating opportunities associated with assortative mating. The observed disruptive selection could cause floral divergence in this population if column and spur lengths are heritable. Further, column and spur lengths are somewhat correlated ( $r=0.544$ ,  $df=54$ ,  $P=0.084$ ), so divergent selection on either of these traits may amplify overall divergent selection on both traits (see Chapter 1 for a more detailed overview).



**Figure 3.1** A *Platanthera dilatata* inflorescence.

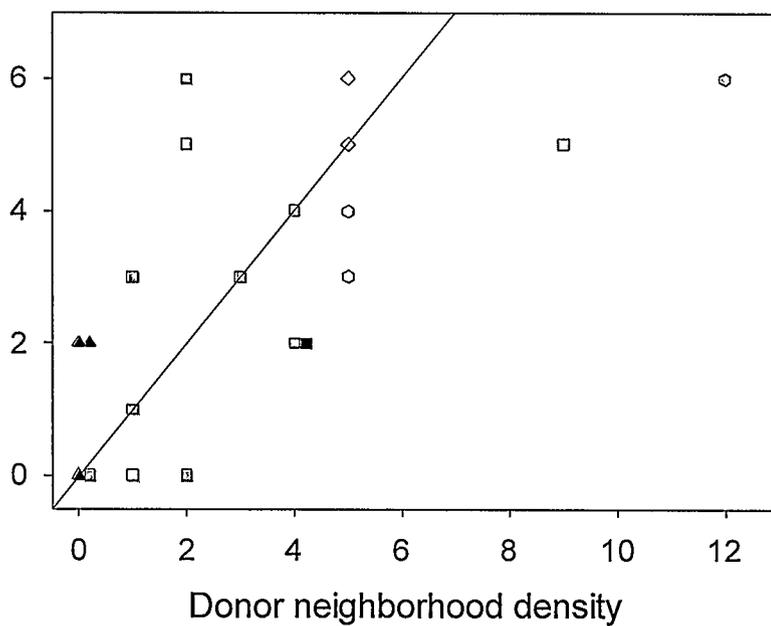
**Red arrows indicate strongly curved nectar spurs, whereas the spurs on other flowers show a variety of spur curvature.**



**Figure 3.2** Observed associations of the floral traits of mating partners in four *Platanthera dilatata* populations.

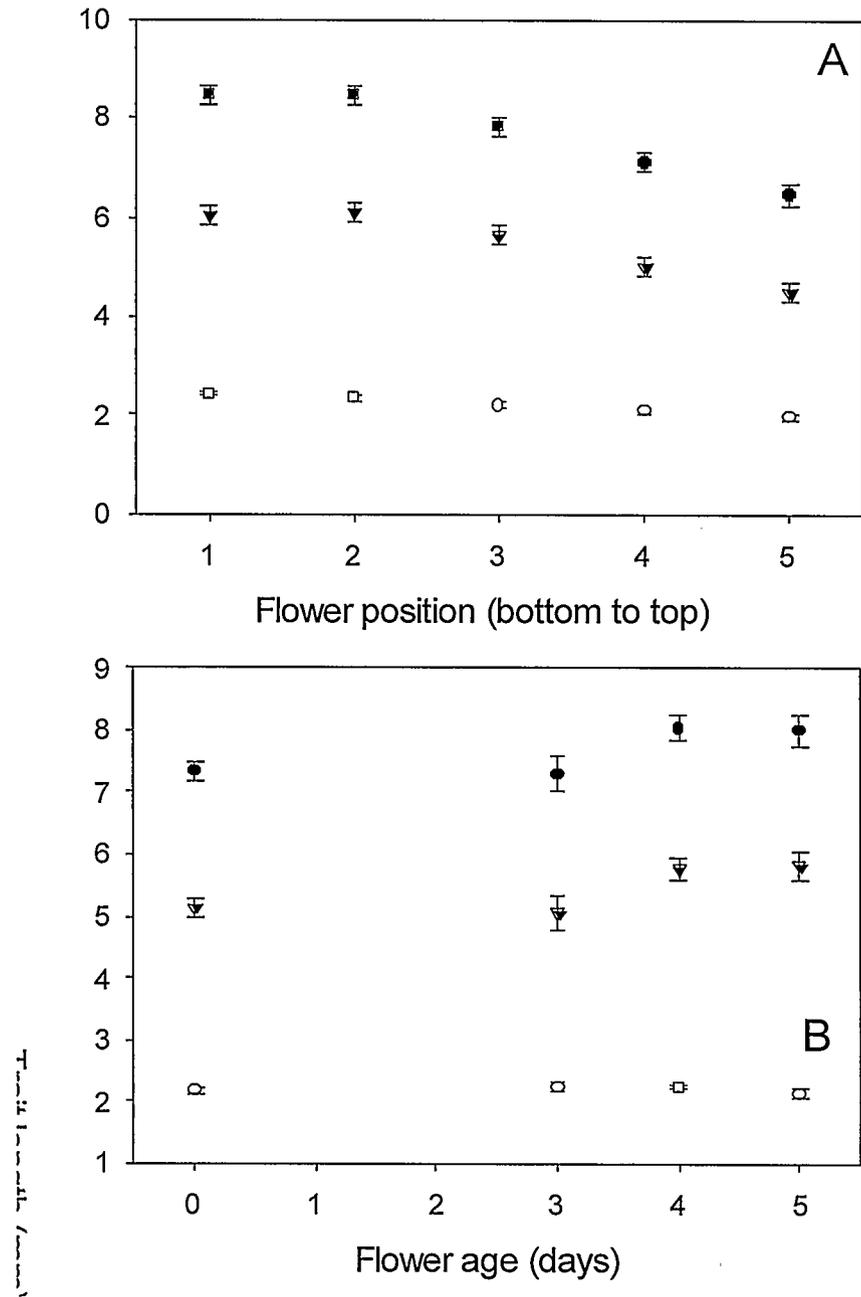
Panels A, C and D depict the relations of column length, spur length and combined column and spur lengths, respectively, of flowers that received pollen from a specific donor plant to the average traits of donor flowers from which pollen was removed.

Panel B illustrates the column lengths of the lowermost nonwilted flowers on recipient and donor plants. Different symbols identify different populations, whereas symbol shading indicates distance between mating pairs (open, <0.1 m; light grey, >0.1 m and <0.5 m; dark grey, >0.5 m and <1 m; black, >1 m). Solid lines identify equal traits of donors and recipients.



**Figure 3.3** Relation of the neighborhood density (plants/0.33 m<sup>2</sup>) of *Platanthera dilatata* plants to that of plants from which they received massulae.

**Different symbols identify different populations, whereas symbol shading indicates distance between mating pairs (open, <0.1 m; light grey, >0.1 m and <0.5 m; dark grey, >0.5 m and <1 m; black, >1 m).**



**Figure 3.4** Relations of mean ( $\pm$  SE) column and spur lengths of *Platanthera dilatata* flowers to A) their position within inflorescences and B) their age.

Different symbols identify different traits: black triangles = spur length; open circles = column length; black circles = column + spur length.

**Table 3.1 Locations (Universal Transverse Mercator coordinates) and elevations for the four *Platanthera dilatata* populations studied for assortative mating.**

Population	Zone	UTM E	UTM N	Altitude (m)
Bostock Lower	11 U	11453128	5675404	971
Bow 1	11 U	11541206	5722867	1935
Bow 2	11 U	11539925	5723215	1969
Bow 4	11 U	11537871	5725186	1959

**Table 3.2 Results of tests of the quadratic effects of column length, nectar-spur length and plant height on male and female performance by *Platanthera dilatata* plants in the Bow 2 population.**

All analyses also considered terms for the linear effects of column and spur lengths and plant height, as well as terms for flower number per plant and the duration of a plant's observation to account for variation in sampling effort. Estimated selection gradients ( $\pm$  SE) are provided for significant effects.

Dependent variable	Sampling distribution	Effect		
		Column	Spur	Height
Probability of pollinarium removal	Binomial	$G_1=4.36^*$ $0.965 \pm 0.649$	$G_1=0.12$	$G_1=0.64$
Pollinarium removal	Normal	$F_{1,44}=0.69$	$F_{1,44}=5.15^*$ $0.207 \pm 0.091$	$F_{1,44}=1.07$
Probability of massula receipt	Binomial	$G_1=4.05^*$ $0.591 \pm 0.315$	$G_1=0.05$	$G_1=0.13$
Massula receipt	Normal	$F_{1,44}=0.58$	$F_{1,44}=2.31$	$F_{1,44}=0.21$
Ovules fertilized	Normal	$F_{1,45}=0.58$	$F_{1,45}=0.01$	$F_{1,45}=0.22$
Seed production	Normal	$F_{1,45}=1.36$	$F_{1,45}=0.07$	$F_{1,45}=0.18$

\* $P < 0.05$

## Chapter Four: Local phenotypic selection on floral traits

### 4.1 Introduction

Adaptive evolution is a central mechanism responsible for the origin and maintenance of biological diversity (Schluter, 2000). In particular, local adaptation caused by dissimilar selection in contrasting environments leads to allopatric divergence in the absence of gene flow (Coyne and Orr, 2004). Even in the face of limited gene flow, local adaptation can promote diversification if long-distance matings produce offspring that perform poorly in either parental environment (outbreeding depression: Waser and Price, 1994). Thus, the occurrence and scale of local adaptation fundamentally influence the opportunity for adaptive diversification.

Local adaptation results from contrasting selection in space, such that individuals have higher fitness in their own habitat than when they grow elsewhere. Reviews by Herrera et al. (2006) and McKay et al. (2005) showed widespread evidence that local adaptation exists at large spatial scales. The studies reviewed by Herrera et al. generally detected local adaptation for populations separated by 4-150 km. However, only seven of studies reviewed showed local adaptation at <4 km. As outbreeding depression has been found for plants separated by as little as 30 m (Waser and Price, 1994), local adaptation may operate at very fine scales.

Angiosperms exhibit extensive floral diversity, including examples of both adaptive radiation (e.g., Barrett and Graham, 1997; Francisco-Ortega et al., 1997; Hodges, 1997) and repeated convergent evolution (e.g., Castellanos et al., 2003). The role of floral traits in mediating precise mating is commonly proposed as a key influence on the evolution of floral diversity. Precise mating should reduce pollen loss during

dispersal (Castellanos et al., 2003), because it increases the chance of pollen being deposited on conspecific stigmas which can further reduce interspecific pollen exchange (Alexandersson and Johnson, 2002). Evidence for this benefit has been observed primarily for species with tubular flowers (Alexandersson and Johnson, 2002; Castellanos et al., 2003) and elongate nectar spurs (Nilsson, 1988; Johnson and Steiner, 1997). Manipulation (specifically the reduction) of these traits reduced the proportion of pollen that reached conspecific plants and receipt of conspecific pollen. The hypothesis that the fit between pollinator and flowers promotes mating precision has motivated various studies of floral diversity (Nilsson, 1988; Hodges, 1997; Alexandersson and Johnson, 2002; Maad and Nilsson, 2004) and it is also commonly invoked as a functional explanation of many floral traits, such as bilaterally symmetric (zygomorphic) flowers and elongate nectar spurs (Bawa, 1995; Waser, 2001; Sargent, 2004). If precise mating promotes diversification of flowering plants, then traits responsible for mating precision are likely targets of selection and should provide widespread examples of local adaptation.

Despite the abundance of phenotypic selection studies for flowering plants, most studies have estimated the strength and direction of phenotypic selection only in one population and during one year (Herrera et al., 2006), so the occurrence and spatial scale of local floral adaptation are poorly understood. Eight out of forty studies reviewed by Herrera (2006) provide some evidence for the occurrence of adaptation on a regional scale. In addition, a study of outbreeding depression in plants have found reduced performance by offspring with parents separated by as little as 30 m (Waser and Price,

1994). The occurrence of contrasting selection on such a local scale has seldom been examined for flowering plants.

In this chapter I assess whether phenotypic selection on floral traits varies among four orchid populations within 0.6-4.2 km of each other. I specifically consider selection on two traits that can influence mating precision, as well as plant height, which is known to affect pollinator behavior (Waddington, 1979). I quantify the effects of these traits on pollen removal and receipt and fruit and seed production to compare the occurrence and extent of directional, stabilizing and disruptive selection among the populations.

## **4.2 Materials and methods**

### ***4.2.1 Field methodology***

I measured phenotypic selection on *Platanthera dilatata* (Pursh) Lindl., a terrestrial orchid that is pollinated primarily by noctuid moths (Boland, 1993). A *P. dilatata* flower produces two pollinaria, each with a single sectile pollinium in which pollen is aggregated into smaller units (massulae), so that pollen from one pollinium can disperse to multiple recipient flowers. Each flower also produces a nectar spur (see Fig. 3.1) and the single anther and pistil are fused into a composite column, so that a flower deposits pollen on and receives pollen from pollinators in the same location.

The four sub-alpine *P. dilatata* populations that I studied are located along Highway 93 in Banff National Park, Alberta of the Canadian Rocky Mountains (see Table 4.1). Adjacent populations are separated from each other as follows: Bow 1 – Bow 2, 1.37 km; Bow 2 – Bow 3, 2.32 km; Bow 3 – Bow 4, 0.6 km. Populations are similar in soil moisture, altitude and aspect (SW).

I measured phenotypic selection on column length, nectar-spur length and plant height for 40 - 90 plants per population, which were selected by stratified random sampling, as described in Chapter 2. To ensure consistent measurement of plant traits, on the first observation day I measured column and spur length on the oldest non-wilting flower with digital calipers and measured plant height with a tape measure. I also estimated four fitness components for these plants: number of flowers that experienced pollinarium removal, number of massulae received, number of fertilized ovules per flower and seed production per flower. Pollinarium removal represents a male fitness component and the remaining responses are female fitness components.

#### ***4.2.2 Data analysis – estimating phenotypic selection***

I estimated the nature and strength of phenotypic selection on floral traits with generalized linear models, as described in Chapter 2. Each analysis included terms for column length, spur length and plant height to assess the general occurrence of phenotypic selection. To test for heterogeneous selection among populations I also included interactions between populations and the three traits. All analyses also included the number of flowers sampled and the observation period for each plant to account for differences in sampling effort.

### **4.3 Results**

#### ***4.3.1 Variation in plant traits and reproductive performance***

Column and spur length showed moderate variation (mean  $\pm$  standard deviation [coefficient of variation];  $2.41 \pm 0.33$  mm [13.9%];  $5.66 \pm 0.99$  mm [17.5%]), whereas plant height varied considerably ( $20.61 \pm 10.99$  cm [53.3%]). Selection can act only on

standing variation within populations, so traits with more among-plant variation provide more opportunity for selection. Thus, the observed coefficients of variation indicate more opportunity for selection on plant height than on spur and column lengths.

All four fitness measures exhibited a many zero observations and right-skewed distribution of outcomes among successful plants (Figure 4.1). Nevertheless, the residuals from the general linear models are largely normally distributed (Figure 4.2).

#### *4.3.2 Phenotypic selection*

All three traits that I measured experienced phenotypic selection, which generally favored taller plants with larger flowers (Tables 4.2 and 4.3). Column length was subject to positive directional selection for the number of pollinaria removed (Table 4.2) and disruptive selection for the probability that a plant had some fertilized ovules (Table 4.3). Spur length provided the most evidence of phenotypic selection of the three traits, largely because of consistent fitness variation among plants that were at least partially successful (i.e., models of continuous, rather than binary outcomes). Spur length experienced positive directional selection of roughly equal magnitude for massula receipt, ovule fertilization and seed production (Table 4.2). In addition, this trait was subject to disruptive selection for massula receipt and stabilizing selection for the probability of ovule fertilization (Table 4.3). The combined effects of directional and disruptive selection on spur length for massula receipt resulted because plants with spurs one standard deviation shorter than the mean had the lowest fitness (Figure 4.3). Finally, plant height experienced positive directional selection for the probability of pollinarium removal, the probability of massula receipt, and the number of massulae received (Table

4.2). Height also experienced stabilizing selection for the probability of ovule fertilization (Table 4.3).

#### ***4.3.3 Variation among populations in reproductive performance***

My analyses detected little fitness variation among populations, after accounting for differences in trait variation (population effects in Tables 4.2 and 4.3). This result is not surprising for fitness measures of plants with some reproductive success, because these measures were centered by each population's trait mean before analysis. Although centering could not be applied to the binary measures of reproductive success or failure, of these fitness measures only the probability of ovule fertilization exhibited significant differences among populations (Tables 4.2 and 4.3).

None of the analyses detected significant differences among populations in the effects of traits on fitness (population x trait interactions;  $P > 0.1$  in all cases). Thus, these populations provide no evidence of local adaptation in column length, spur length or plant height.

### **4.4 Discussion**

#### ***4.4.1 Overall phenotypic selection***

Column length experienced less selection than spur length, even though column and spur lengths are proposed to be equally important in promoting precise mating by reducing pollen wastage (see Chapter 3). Column length may have experienced less selection because it varies less than spur length (Cresswell, 1998), and therefore has less opportunity for selection. However, this finding is surprising because column size (length and width) of *Platanthera* has been shown to increase the precision of pollen placement

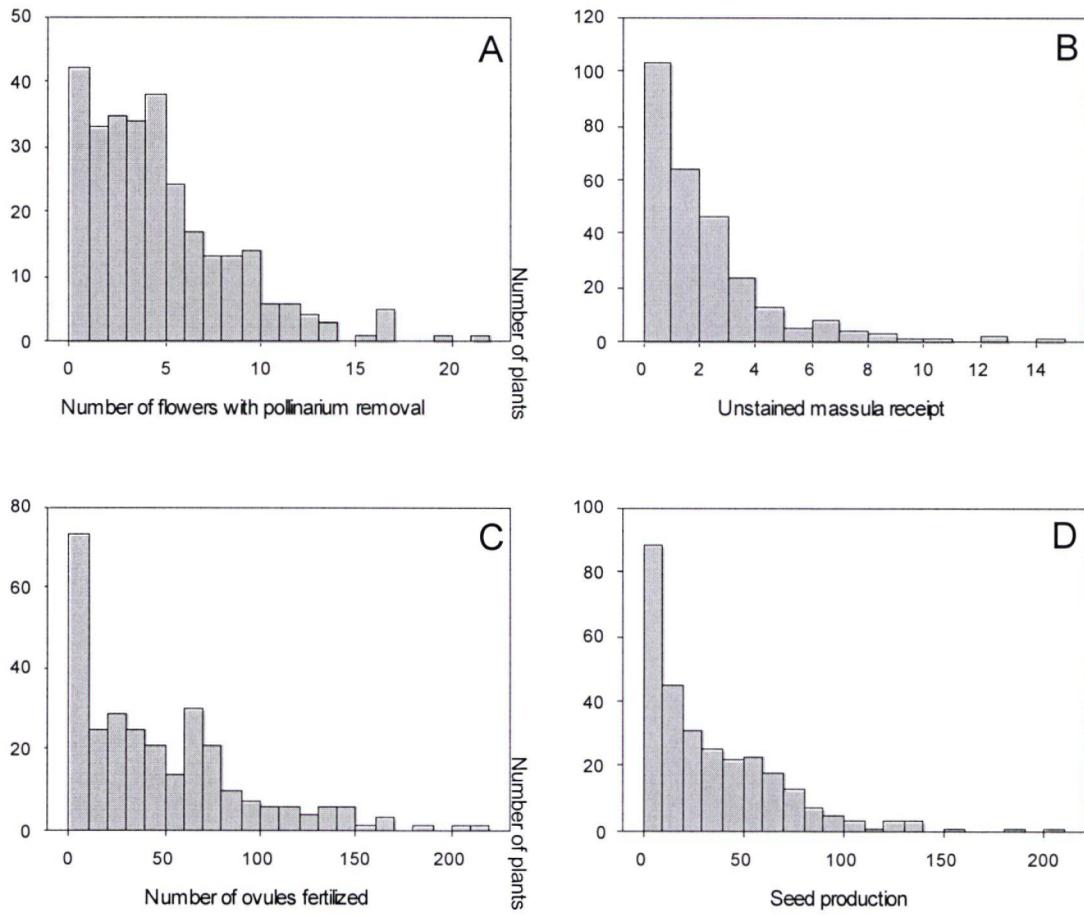
(Maad and Nilsson, 2004), and so should increase pollen export efficiency. This result leads to the expectation that column length should be under strong selection, because it can increase fitness. Furthermore, role organ length in promoting mating precision by governing the fit between pollinators and flowers should apply for monomorphic plants (Cresswell, 1998), as has been demonstrated for heterostylous species (Eckert and Barrett 1994b). Perhaps the observed limited selection for column length resulted from a variable pollinator environment. Such variation could result in conflicting selection that tends to cancel out. For example, *Platanthera dilatata* is primarily pollinated by insects that differ in tongue length (skippers and noctuid moths) (Boland, 1993), thus each pollinator can exert contrasting selection on column length if the column interacts differently with different pollinators. This hypothesis could be tested with pollinator-exclusion experiments to determine whether pollinators cause contrasting selection.

Flower traits that determine the depth that nectar-collecting pollinators must probe often experiences strong selection, as others have found in tubular flowers (Campbell, 1991; Johnston, 1991; Caruso, 2001) and spurred orchids (Johnson and Steiner, 1997). My findings are congruent with these studies, as I found frequent selection for spur length. However, in the absence of assortative mating for spur length (see Chapter 3), the resultant selection could not have resulted from assortment and must be caused by some other, unidentified, process.

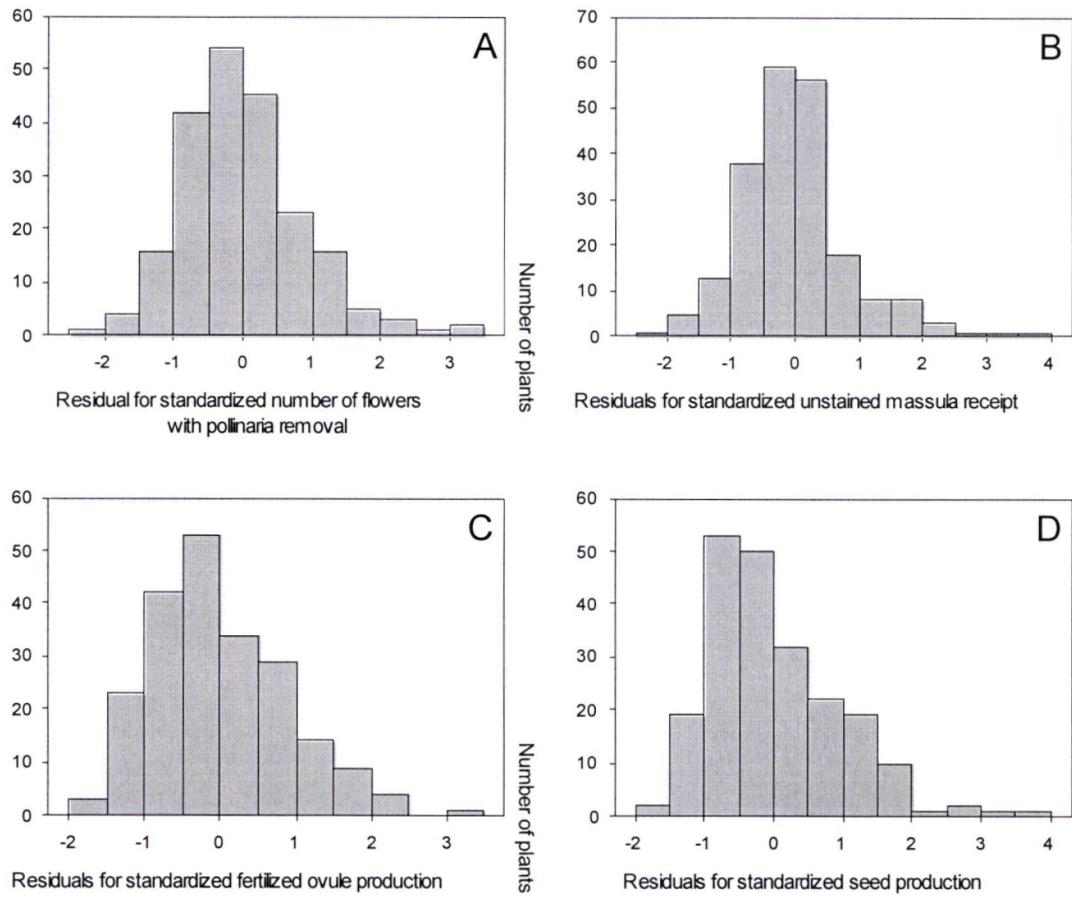
Plant height experienced a similar incidence of selection as spur length. Plant stature can affect mating precision (Waddington, 1979) and be involved in assortative mating (Waddington, 1979). Thus, pollinator behaviour could have caused the observed combination of stabilizing selection and positive directional selection.

#### ***4.4.2 Among-population variation in selection***

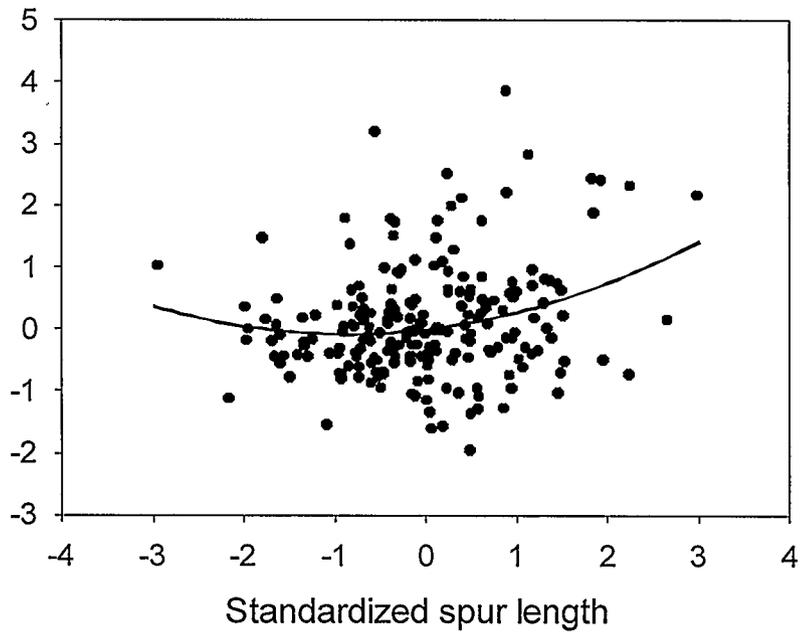
If local adaptation occurs at very small spatial scales for *P. dilatata*, I should have observed heterogeneous selection among populations. The lack of significant population x trait interaction for all three traits at the spatial scale of my study contradicts this expectation. Others have detected contrasting selection for plant populations separated by <4 km (Gilbert et al., 1996; Campbell et al., 1997; Caruso, 2000; Irwin, 2000; Schemske and Bierzychudek, 2001; Totland, 2001; Aspi et al., 2003). Such differences require contrasting environmental conditions (e.g. altitude, aspect, soil moisture) that induce dissimilar relations of performance to trait variation among populations. Previous studies that found selection at small spatial scales (<4 km) often involved altitudinal differences of 250 m (Gomez, 1993; Totland, 2001), which can influence temperature and thus community structure. Community structure can cause contrasting selection among flowering plants, because of variation in the pollinator fauna (O'Connell and Johnston, 1998), competition with other species for pollinators (Caruso, 2000) and variation of intensity of herbivory (Gomez and Zamora, 2000). In contrast to such studies, the four populations that I studied differed in elevation by at most 34 m. Thus, the homogeneous selection that I observed suggests similar pollination environments in all four populations.



**Figure 4.1** Frequency distribution of overall fitness components for four *Platanthera dilatata* populations.



**Figure 4.2** The frequency distributions of residuals of standardized fitness components from the general linear models for plants that reproduced successfully.



**Figure 4.3** An example of disruptive selection on standardized spur length for the number of massulae received by individual *Platanthera dilatata* plants.

**Table 4.1** Locations and elevations of *Platanthera dilatata* populations studied for phenotypic selection.

Population	Zone	UTM E	UTM N	Altitude (m)
Bow 1	11 U	11541206	5722867	1935
Bow 2	11 U	11539925	5723215	1969
Bow 3	11 U	11537978	5725013	1956
Bow 4	11 U	11537871	5725186	1959

**Table 4.2 Results of tests of the linear effects of column length, nectar-spur length and plant height on male and female performance by *Platanthera dilatata* plants in four closely spaced populations.**

**All analyses also considered terms for flower number per plant and the duration of a plant's observation to account for variation in sampling effort. Estimated selections gradients ( $\pm$  SE) are provided for significant effects.**

Dependent variable	Sampling	Effect			
	distribution	Population	Column	Spur	Height
Probability of pollinarium removal	Binomial	$G_3=0.55$	$G_1=0.42$	$G_1=0.53$	$G_1=6.84^{**}$ $0.601 \pm 0.243$
Pollinarium removal	Normal	$F_{3,203}=0.16$	$F_{1,203}=6.16^*$ $0.165 \pm 0.066$	$F_{1,203}=0.35$	$F_{1,203}=0.37$
Probability of massula receipt	Binomial	$G_3=2.25$	$G_1=2.83$	$G_1=0.23$	$G_1=15.06^{***}$ $0.599 \pm 0.163$
Massula receipt	Normal	$F_{3,204}=0.26$	$F_{1,204}=3.66$	$F_{1,204}=6.85^{**}$ $0.179 \pm 0.068$	$F_{1,204}=4.94^*$ $0.157 \pm 0.070$
Probability of ovule fertilization	Binomial	$G_3=9.20^*$	$G_1=2.63$	$G_1=2.11$	$G_1=0.64$
Ovules fertilized	Normal	$F_{3,205}=1.18$	$F_{1,205}=0.28$	$F_{1,205}=4.54^*$ $0.148 \pm 0.070$	$F_{1,205}=2.02$
Seed production	Normal	$F_{3,205}=0.92$	$F_{1,205}=0.05$	$F_{1,205}=3.99^*$ $0.145 \pm 0.072$	$F_{1,205}=1.77$

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

**Table 4.3 Results of tests of the quadratic effects of column length, nectar-spur length and plant height on male and female performance by *Platanthera dilatata* plants in four closely spaced populations.**

All analyses also considered terms for the linear effects of column and spur lengths and plant height and flower number per plant and the duration of a plant's observation to account for variation in sampling effort. Estimated selections gradients ( $\pm$  SE) are provided for significant effects.

Dependent variable	Sampling distribution	Effect			
		Population	Column	Spur	Height
Probability of pollinarium removal	Binomial	$G_3=0.54$	$G_1=0.05$	$G_1=1.22$	$G_1=0.17$
Pollinarium removal	Normal	$F_{3,200}=0.25$	$F_{1,200}=1.22$	$F_{1,200}=3.35$	$F_{1,200}=0.01$
Probability of massula receipt	Binomial	$G_3=2.26$	$G_1=0.67$	$G_1=1.59$	$G_1=2.85$
Massula receipt	Normal	$F_{3,201}=0.34$	$F_{1,201}=0.79$	$F_{1,201}=4.67^*$ $0.099 \pm 0.046$	$F_{1,201}=0.71$
Probability of ovule fertilization	Binomial	$G_3=17.92^{***}$	$G_1=4.56^*$ $0.382 \pm 0.194$	$G_1=4.93^*$ $-0.263 \pm 0.127$	$G_1=13.12^{***}$ $-0.437 \pm 0.137$
Ovules fertilized	Normal	$F_{3,202}=1.08$	$F_{1,202}=2.96$	$F_{1,202}=0.87$	$F_{1,202}=0.75$
Seed production	Negative Binomial	$F_{3,202}=0.85$	$F_{1,202}=1.98$	$F_{1,202}=0.43$	$F_{1,202}=0.37$

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

## Chapter Five: Concluding Discussion

### 5.1 Effects of within-plant variation on assortative mating

Within-individual trait variation can strongly influence plant-level processes such as precise mating among individuals (Bateman and Rudall, 2006). A survey of seven European orchids (including a *Platanthera* species) found that floral morphology commonly varied significantly with flower position on inflorescences (Bateman and Rudall, 2006). This observation is not congruent with current hypotheses of how orchid floral traits, such as elongate nectar spurs and fused reproductive organs, cause mating precision and promote pollinator specialization and reproductive isolation (Nilsson, 1988; Johnson and Steiner, 1997; Maad and Nilsson, 2004). To function in this manner, mating would have to be precise enough to overcome the standing within-plant variation to generate the remarkable orchid floral diversity. Mating precision can promote successful pollen exchange (Alexandersson and Johnson, 2002), but within-individual trait variation can increase the incidence of unsuccessful pollen exchange by reducing mating precision. Analytical models would be the logical next step to study how precise mating must be to overcome the diluting effects of within-plant variation. Further, to understand the extent of trait variation within individuals, characters in plants that have been shown to influence reproductive fitness (style and stamen length) should be surveyed. Because plants are modular and within-plant trait variation seems ubiquitous, this aspect of reproductive specialization caused by mating precision should not be ignored.

## 5.2 Flower-level mechanisms for assortative mating

Column and spur lengths have been proposed to function together to cause precise mating (Nilsson, 1988; Johnson and Steiner, 1997; Maad and Nilsson, 2004). The lengths of reproductive organs are important in placing pollen precisely on pollinators. For example, flowers of heterostylous species, with reciprocal lengths of styles and stamens between morphs, mate disassortatively (Barrett, 1990; Eckert and Barrett, 1992; Eckert and Barrett, 1994a; Eckert and Barrett, 1994b; Barrett, 2002). This mechanism results because these organs influence where pollinators carry pollen on their bodies. Logically, this relation should lead to positive assortment among flowers of monomorphic species (Cresswell, 1998). The floral-level assortative mating by column length that I observed (Figure 3.2A) is evidence for this mechanism. However, I found no evidence for assortative mating by spur length.

Spur length (or corolla-tube depth) are thought to control how pollinators interact with flowers (Nilsson, 1988; Hodges and Arnold, 1994; Hodges, 1997; Johnson and Steiner, 1997; Castellanos et al., 2003; Castellanos et al., 2004). For example, Johnson and Steiner (1997) found that spur lengths control how pollinators deposit pollen onto stigmas in another moth-pollinated orchid, *Disa cooperi*, and that plants with artificially shortened spurs produced fewer seeds. Current evidence for the effects of nectar spurs on mating precision and efficiency does not account for within-plant variation in spur length, although given low within-plant variation in spur length, the proposed mechanism of pollinator fit with floral traits should still lead to flower-level precise mating. One explanation for the lack of assortment by spur length in my study is the hypothesis concerning variation in spur curvature, which I outlined in Chapter 3. Alternatively,

some aspects of pollinator behavior may affect how the *P. dilatata* flowers interact with pollinators.

If spur length promotes precise mating in long-tongued insect-pollinated orchids with spurs (such as *Disa cooperi*, Johnson and Steiner, 1997) then the interaction of *P. dilatata* with its pollinators must differ from that of orchids for which this effect has been demonstrated. Such effects could be studied by observing the behaviour of visitors to *P. dilatata* flowers to determine whether nectar spurs constrain their contact with viscidia and stigmas. This may prove difficult for *Platanthera dilatata*, because the moths that visit these plants are active only during almost complete darkness. In addition, low pollinator activity (~ 3 hrs. of observation yielded 1 floral visitor; 8-9 days of exposure to pollinators resulted in an average of 5.9 pollinaria removed per plant) make consistent observation of pollinators difficult. One way to address this is to use night-vision photographic equipment to capture pollinator visits; however, this does not resolve the problem of low pollinator activity.

### **5.3 Local phenotypic selection**

The four closely-spaced populations that I studied seem to experience similar phenotypic selection on floral traits, whereas other studies have detected variation in selection and local adaptation at the scale of 0.2 – 3.5 km (Gomez, 1993; Totland, 2001). Overall, most evidence for spatial variation in selection involves comparisons between more widely separated populations (McKay et al., 2005; Herrera et al., 2006). As distances increase between populations, there is a higher likelihood that some feature of the environment will differ between populations. In most of the studies that found

variation in selection among closely situated populations, ecological factors other than linear distance were identified as causal factors. Spatial distance between populations can be a rough correlate of variation in the causal mechanism for selection, but as the spatial scale decreases, the environmental contrast can become indistinguishable.

In summary, my thesis provides the first direct evidence for positive assortative mating among flowers caused by reproductive organ length. However, I did not find similar patterns at the plant level, likely owing to large within-plant variation in floral traits. I did not find evidence of stabilizing selection on the trait causing assortment as predicted by Kirkpatrick and Nuismer (2004), probably because of the lack of plant-level assortment or other sources of selection. Additionally, despite considerable evidence of phenotypic selection on floral traits, I did not detect contrasting selection among closely positioned populations, likely because of environmental similarity among populations.

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