INVESTMENT AND ALLOCATION OF NECTAR PRODUCTION IN AN ANIMAL-POLLINATED PLANT

by

Jay M. Biernaskie
B.Sc. Biological Sciences, University of Lethbridge 2001

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

In the
Department of
Biological Sciences

SIMON FRASER UNIVERSITY

Fall 2004

APPROVAL

Name: Jay Michael Biernaskie

Degree: Master of Science

Title of Thesis:

Investment and allocation of nectar production in an animal-pollinated plant.

Examining Committee:

Chair: Dr. J.M. Webster

Dr. E. Elle, Assistant Professor
Department of Biological Sciences, S.F.U.

Dr. B.D. Roitberg, Professor
Department of Biological Sciences, S.F.U.

Dr. R.C. Ydenberg, Professor
Department of Biological Sciences, S.F.U.

Dr. C.G. Eckert, Associate Professor
Department of Biology, Queen's University
Public Examiner

October 8th, 2004
Date Approved

SIMON FRASER UNIVERSITY

PARTIAL COPYRIGHT LICENCE

The author, whose copyright is declared on the title page of this work, has granted to Simon Fraser University the right to lend this thesis, project or extended essay to users of the Simon Fraser University Library, and to make partial or single copies only for such users or in response to a request from the library of any other university, or other educational institution, on its own behalf or for one of its users.

The author has further granted permission to Simon Fraser University to keep or make a digital copy for use in its circulating collection.

The author has further agreed that permission for multiple copying of this work for scholarly purposes may be granted by either the author or the Dean of Graduate Studies.

It is understood that copying or publication of this work for financial gain shall not be allowed without the author's written permission.

Permission for public performance, or limited permission for private scholarly use, of any multimedia materials forming part of this work, may have been granted by the author. This information may be found on the separately catalogued multimedia material and in the signed Partial Copyright Licence.

The original Partial Copyright Licence attesting to these terms, and signed by this author, may be found in the original bound copy of this work, retained in the Simon Fraser University Archive.

W. A. C. Bennett Library
Simon Fraser University
Burnaby, BC, Canada

ABSTRACT

A plant's ability to attract animal pollinators should be influenced by two important factors: the amount of available resources that is invested to the production of rewards such as nectar, and the individual's size or resource state. Using *Chamerion* (= *Epilobium*) *angustifolium* (Onagraceae) as a model, I investigate: (1) selection pressures that determine the evolutionarily stable investment to nectar production, and (2) allocation tactics that vary with an individual's current resource state. A conceptual model is presented, in which a plant's attractiveness to pollinators increases with relative investment to nectar production ('social competition'). The evolutionarily stable investment strategy occurs where the expense of further nectar production outweighs the potential disadvantage of being slightly less attractive than competitors. The model may explain why *C. angustifolium* inflorescences produce, on average, such large amounts of nectar. Within populations, however, individuals varied greatly in the amount of nectar offered to pollinators, mainly due to variation in floral display size (number of open flowers—a correlate of resource state). Given that large, attractive inflorescences maximize pollen export by limiting the amount of pollen removed by each visitor, I predicted a size-dependent distribution of nectar within the vertical inflorescences of *C. angustifolium*. As predicted, small inflorescences distributed nectar nearly evenly among (lower) female- and (upper) male-phase flowers, whereas larger inflorescences allocated extra nectar to female-phase flowers. In experimental inflorescences, I distributed the same volume of nectar to mimic the 'large' and 'small' allocation types. Nectar-foraging bumblebees visited a mean of 3.2 fewer male-phase flowers on the 'large', relative to 'small' type, as expected if the nectar distribution of large displays functions to limit pollen removal during individual visits. I propose that the nectar gradient may adaptively mediate the schedule of pollen removal by manipulating the patch departure behaviour of pollinators.

ACKNOWLEDGEMENTS

Many thanks go to my thesis committee: Elizabeth Elle, Bernie Roitberg, and Ron Ydenberg; I greatly appreciate your insights and enthusiasm for my work. I am especially grateful to E. Elle for financial support, friendly mentorship, and for endless encouragement to pursue my interests; I have become a much better scientist under your direction. Thanks to Chris Eckert for being at the defence and for many helpful comments on the thesis.

Many friends and colleagues have made my experience at SFU an excellent one. Thanks to my labmates, Sandy Gillespie and Mike Stastny for much appreciated entertainment. Mathias Schuetz and Samantha Vibert helped to make my field seasons a lot less stressful than they could have been; Kathleen Takeda was extremely helpful in the lab. Thanks to the faculty and students of BERG for such a great atmosphere to be part of, including Eric Davies, Joel Heath, Karl Laroche, Patrik Nosil and Maxence Salomon for extra-curricular activities. Larry Dill was always available for helpful discussions. Jennifer Perry was indispensable for editing and formatting, and was a great friend throughout my time at SFU.

I am thankful for funding from the Natural Sciences and Engineering Research Council of Canada and the Dean of Graduate Studies.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1 GENERAL INTRODUCTION

Attraction of insect pollinators is a critical component of fitness for most entomophilous angiosperms. Obviously, pollinator visits are necessary to import enough pollen to fertilize ovules and to effectively disperse most of a plant's own pollen; a frequent visit rate is particularly critical, however, for the latter function (reviewed by Harder, et al., 2001). Because of diminishing returns associated with increased pollen removal by each visitor, pollen export is maximal when many different pollinators disperse only small amounts of pollen at a time (Harder and Thomson, 1989, Iwasa, et al., 1995). Thus, selection should favour phenotypes that attract a large number of animal pollinators, as long as each individual pollinator removes only a limited amount of pollen.

A plant's ability to attract animal pollinators should be affected by both genetic and environmental influences. Presumably, the amount of available resources that is invested to pollinator attraction (the investment strategy) is subject to genetic variation, and hence natural selection. But plants within a population differ greatly in total resources available (resource state), and this variation is likely influenced by the quality of environment. In this thesis, I investigate selection pressures that determine the evolutionarily stable investment to attractive phenotypes, and mechanisms that plants might use to maximize fitness, depending on resource state.

I focus on nectar production phenotypes of *Chamerion angustifolium* (Onagraceae). Plants of this species are, on average, particularly attractive to bumblebee pollinators, which is attributable to a high mean rate of floral nectar production (e.g. Heinrich, 1979). Inflorescences vary widely in the number of rewarding flowers presented on a daily basis (and hence attractiveness to pollinators), however, and this variation appears positively correlated with ramet size. Nectar production is distributed within the inflorescence in a gradient that decreases from bottom (female-phase flowers) to top (male-phase flowers; Pyke, 1978). This pattern is generally understood as an adaptation to increase the efficiency of cross-pollination (e.g. Best and Bierzychudek, 1982). In chapters 2 and 3 respectively, I address two key questions: (1) why, on average, do individuals invest so much energy into nectar production? and (2) how should plants distribute nectar among flowers, depending on attractiveness to pollinators?

Throughout the thesis, my goal is to elucidate the kinds of selection that have shaped existing phenotypes. Following Reeve and Sherman (1993), I consider an adaptation as "a phenotypic variant that results in highest fitness among a specified set of variants in a given environment" (p. 67). For practical reasons, I typically consider a trait's functional design as the criterion for fitness. I follow two typical approaches to the analysis of functional design (from Thornhill, 1990). First, to investigate the function of a presumed adaptation, one may compare the functional consequences ('fitness') of phenotypic variants, either theoretically or experimentally. Natural selection theory predicts that among a specified set of variant phenotypes, the most adapted (functional) phenotype will be the one that predominates in a given environment (see Reeve and Sherman, 1993). Second, one may recognize the need for an adaptation to deal with a specified ecological problem. This leads to a hypothesis of the mechanisms that might solve the problem, and a prediction of the phenotype that should predominate in a given environmental context. Specific examples of both approaches are given below.

In Chapter 2, I explore the selection pressures that determine the evolutionarily stable investment to nectar production. I argue that the best investment strategy to adopt may depend on the strategies of competing plants in the population. A model is presented that includes the mean investment phenotype of competitors as part of the environmental context in which alternative phenotypes are compared. If an individual's access to pollinators is negatively related to the rewards offered by competitors, then an extravagant investment to nectar production may be adaptive. I report the results of nectar addition experiments that were designed to investigate the mechanisms of competitive interaction among *C. angustifolium* inflorescences.

In chapter 3, I consider facultative adjustment of the nectar distribution in *C. angustifolium*, depending on attractiveness to pollinators. Based on models of pollen export maximization, I suggest that the largest ramets in the population (those with the largest floral displays) should limit per-visit pollen removal more than smaller, less attractive conspecifics. I predict that the strength of the nectar gradient should vary positively with the number of flowers displayed, to adaptively mediate the number of pollen-bearing flowers probed per bumblebee visit. To further clarify the function of the nectar distribution, I use controlled experiments in the field to compare the consequences of variant nectar distributions on bumblebee foraging behaviour and expected pollen removal. I expect that the distribution phenotype observed in the largest displays should be adaptive only in the context of large floral displays (i.e. frequent pollinator visits), and vice versa.

LITERATURE CITED

Best, L.S. and Bierzychudek, P. 1982. Pollinator foraging on foxglove (*Digitalis purpurea*): A test of a new model. *Evolution*, **36**: 70-79.

Harder, L.D. and Thomson, J.D. 1989. Evolutionary options for maximizing pollen dispersal in animal-pollinated plants. *American Naturalist*, **133**: 323-344.

Harder, L.D., Williams, N.M., Jordan, C.Y. and Nelson, W.A. 2001. The effects of floral design and display on pollinator economics and pollen dispersal. In *Cognitive Ecology of Pollination* (Chittka, L. and Thomson, J.D., eds), pp. 297-317. Cambridge University Press.

Heinrich, B. 1979. *Bumblebee Economics.* Harvard University Press.

Iwasa, Y., de Jong, T.J. and Klinkhamer, P.G.L. 1995. Why pollinators visit only a fraction of the open flowers on a plant: The plant's point of view. *Journal of Evolutionary Biology*, **8**: 439-453.

Pyke, G.H. 1978. Optimal foraging in bumblebees and coevolution with their plants. *Oecologia*, **36**: 281-293.

Reeve, H.K. and Sherman, P.W. 1993. Adaptation and the goals of evolutionary research. *Quarterly Review of Biology*, **68**: 1-32.

Thornhill, R. 1990. The study of adaptation. In *Interpretation and Explanation in the Study of Animal Behaviour* (Bekoff, M. and Jamieson, D., eds), pp. 31-62. Boulder: Westview Press.

CHAPTER 2 SOCIAL COMPETITION IN FLOWERING PLANTS: A POLLINATOR ATTRACTION GAME

ABSTRACT

I explore an evolutionary game among animal-pollinated plants, in which access to a limited pollinator resource increases with relative investment to reward production ('social competition'). I demonstrate how a costly investment strategy may evolve even as the mean fitness of individual plants in the population declines. The ESS occurs where the cost of further nectar production outweighs the potential disadvantage of being slightly less attractive than competitors. In a population of *Chamerion angustifolium*, I used nectar addition experiments to determine how access to bumblebee pollinators depends on a plant's relative nectar availability. Immediately after visiting a nectar-enriched patch of inflorescences, bumblebees probed *more* flowers per (non-enriched) inflorescence and visited a *greater* number of available inflorescences, relative to a control environment. This 'over-visitation' diminished with time, however, as bees apparently learned the location of my small-scale manipulations. I discuss the limitations of these experiments and of available evidence related to the influence of social competition on reward phenotypes. Lastly, I highlight the significance of social competition theory for current conceptions of sexual selection in flowering plants.

INTRODUCTION

How much energy should flowering plants invest into characters that reward their animal pollinators? It depends on the environmental context in which alternative reward phenotypes are evaluated (cf. Reeve and Sherman, 1993). Presumably, one particularly important selective agent in the plant's environment is the existence of conspecifics that compete for effective pollinator service. The competitive interaction for pollinator service and its implications for adaptive reward production are rarely considered, however. Here, I investigate interactions among nectar-producing plants to explore the function of reward phenotypes in a game-theoretic context.

Investment to nectar production may determine the rate of pollinator visits to a plant and the number of flowers probed per visit (refs. in Klinkhamer and deJong, 1993). A frequent visitation rate should be particularly beneficial for pollen dispersal, provided that plants have mechanisms to limit the amount of pollen removed by each individual visitor (reviewed by Harder et al., 2001, see Chapter 3). Nevertheless, the benefit of nectar production may eventually reach an asymptote, if further investment eventually leads to excess pollen removal per visit (Fig.1a). Based on a similar mechanistic argument, some authors have suggested that plants should not produce 'too much' nectar (e.g. Klinkhamer and deJong, 1993, Klinkhamer et al., 1994). But consider that a plant's attractiveness to optimally foraging pollinators depends on a comparison with other plants in the population (Pyke, 1980). In this light, the success of a particular reward phenotype depends not so much on the absolute investment, but on the rewards offered by competing plants (see also Sakai, 1993, Thakar et al., 2003).

Intraspecific competition for pollinator service may exemplify an evolutionary game, in which access to a limited resource increases with relative investment to competitive ability (herein 'social competition', see also Abrams and Matsuda, 1994, Maynard Smith and Brown, 1986, Parker, 1983, West-Eberhard, 1983). Under social competition, the optimal investment to nectar production escalates as the mean investment of the population rises. Nectar is energetically costly to produce, however, and so the mean fitness of plants in the population declines as progressively extravagant phenotypes are favoured (Fig. 1b). (Here 'extravagant' refers to a strategy that is more costly than that which would maximize population mean fitness). The ESS (evolutionarily stable strategy, Maynard Smith, 1982) occurs where the expense of further nectar production (reflecting some life-history trade-off) outweighs the potential disadvantage of being slightly less attractive than competitors. This is, explicitly, the point at which plants should not produce too much nectar.

Several previous approaches have predicted an ESS investment to nectar rewards as a component of the sex allocation strategy (e.g. Charlesworth and Charlesworth, 1987, Charnov and Bull, 1986, Morgan, 1992). Sex allocation problems require a game-theoretic approach because the within-gender competition for mating success is necessarily frequency-dependent (Charnov, 1982, pp. 8-9). In the models cited above, however, the success of a particular reward phenotype is *independent* of the rewards offered by competitors. When social competition for pollinator service is also considered, the ESS investment to rewards may be considerably greater than current sex allocation models predict.

Mechanisms of social competition among animal-pollinated plants

There are two plausible mechanisms by which a plant's access to pollinators may be limited by competition with more rewarding conspecifics (from Sakai, 1993). First, pollinators may learn the location of relatively rewarding plants (see Thomson, 1988) and return to those at the expense of others. Second, assume that, on average, pollinators encounter a greater reward per visit to plants with relatively high rates of nectar production, all else being equal. Pollinators may then visit fewer flowers on ('reject') less rewarding plants than they would if not initially rewarded. Similarly, nectar foragers may quickly pass through patches of relatively unrewarding plants.

An important challenge for empiricists is to understand the extent to which a plant's access to pollinators depends on its relative reward phenotype, via one of the mechanisms above. One useful approach may be to alter the reward phenotype of some individuals, while monitoring pollinator responses to locally competing plants. By comparing pollinator responses in the presence and absence of the altered phenotype, one may gain insight into the extent of competitive interaction among plants.

Here, I determine the effect of an experimentally enriched nectar environment on the subsequent sampling behaviour of nectar-foraging bumblebees (mechanism 2, above) in populations of *Chamerion angustifolium* (Onagraceae). I ask:

- ☐ how does bumblebee visitation to non-enriched inflorescences change after visitation to a relatively rewarding patch of inflorescences?
- ☐ how is this effect altered with bumblebee experience in the enriched environment?

METHODS

I studied the behaviour of nectar-foraging worker bumblebees (*Bombus* spp., mostly *B. flavifrons* and *B. bifarius*) in large patches of *Chamerion (=Epilobium) angustifolium* (L.) Holub. Flowers produce large amounts of nectar relative to other species that are commonly visited by bumblebees (Heinrich, 1979), especially when in female-phase (positioned at the bottom of the vertical inflorescence). The largest ramets in a population allocate especially large amounts of nectar to female-phase flowers, and may do so as an alternative to total flower production (Chapter 3).

All experiments were performed in the clearings of Silver Star Resort, near Vernon, British Columbia, Canada (50.3° N, 119.3° W) in the summer of 2003. In one set of experiments, I observed presumably 'naïve' bumblebees (*short-term responses*); in the second set, I allowed sufficient time for bees to return to a nectar-enriched environment (*long-term responses*).

Short-term responses

The immediate response of bumblebees to an enriched environment was examined with an array experiment. Sixteen linear arrays of three 'stations' each were constructed. A station was made up of two intact, adjacent inflorescences tied together in a v-shape, with inflorescence tips ~20 cm apart. The mean distance between stations was 31.6 cm (std. dev.= 6.3 cm). Each inflorescence within an array was trimmed to display the same number of flowers, and all other inflorescences within ~2 m of the array were clipped off and discarded. Arrays were constructed so that bumblebees might forage uni-directionally through the array, and stations were numbered accordingly, where 'station 1' was the first station visited in the array.

At each array, three control trials and three nectar-enriched trials were assigned in random order. A trial consisted of a foraging bout in which a bumblebee probed at least 5 flowers at station 1 and then visited both stations 2 and 3, in that order. For nectar-enriched trials, I added 2 µl of 42% sucrose solution ('nectar') to the six lowermost flowers of the inflorescences at station 1. (This concentration was equal to the mean of a sample of flowers measured in the field, and the volume greatly exceeded what bees normally encounter on non-enriched inflorescences). Bees usually emptied all nectar-enriched flowers; however, any missed flowers were drained with a clean 2µl microcapillary tube at the end of the trial. For the first 10 minutes following a trial, bees were discouraged from visiting the array with a covering of bridal veil or with a gentle back-hander. Hence, the flowers of stations 2 and 3 (and the flowers of station 1 during a control trial) always contained at least the amount of nectar produced in 10 minutes. During a trial, I recorded the number of flowers probed at each inflorescence and the position (station number) of each visited inflorescence.

Analysis. First, I considered whether the number of flowers probed per inflorescence at stations 2 and 3 depended on the reward quality of station 1. I calculated the mean number of flowers probed on each inflorescence before first departure for each array-station-treatment level combination (n = 64). My linear model included two main factors and their interaction: 'nectar environment', assigned to the array, and 'station number', acting within each array. Both levels of nectar environment (control and nectar-enriched) appeared once in each array, and thus, I used 'array' as a (random) blocking factor. Finally, an array-by-nectar environment term was included to represent the experimental units at the array level.

Second, I tested whether the number of inflorescences visited in the array (after station 1) varied with nectar environment. For each array (n = 16), I calculated the difference between the mean number of inflorescences visited per nectar-enriched trial and the mean number visited per control trial. The effect of nectar environment was evaluated with a (two-tailed) paired t-test.

Long-term responses

Many nectar-collecting bumblebees in a large population of *C. angustifolium* follow regular foraging routes ('traplines') throughout the day (JMB, pers. obs.). Bumblebee responses to an enriched environment may therefore change over time, as individuals track changes in their environment.

I observed the departure behaviour of bumblebees in an enriched environment for four consecutive hours. Two intact, adjacent inflorescences (30-60 cm apart) were trimmed to display the same number of flowers, and one was assigned to a nectar-enriched treatment (same as above). During the observation period, I recorded the number of flowers probed by each visiting bee to either of the two focal inflorescences. After each visit to the nectar-enriched plant, I promptly replenished nectar levels of the bottom six flowers. This procedure was repeated at eight inflorescence pairs.

Analysis. Data were summarized in hourly intervals. At each inflorescence pair, I calculated the mean number of flowers probed during: (1) visits to the nectar-enriched inflorescence, (2) visits to the control inflorescence after first visiting the nectar-enriched neighbour (E→C), and (3) visits to the control inflorescence before visiting the nectar-enriched neighbour (C→E). The mean number of flowers probed for each visit type was estimated while controlling for 'inflorescence pair' (random).

I also tested whether the rate of visitation to the focal inflorescences changed over time. The interest here was not the absolute visit frequency, but whether a shift in visit frequency to one inflorescence type was associated with a shift in visit frequency to the other type. For each hour, and at each inflorescence pair, I calculated the difference between the number of visits to the E inflorescence and number of visits to the C inflorescence. I used an ANOVA model to test for variation among the hourly differences, which included the factors 'inflorescence pair' (random) and 'hour'.

All data were analyzed with JMP 4.0 (SAS Institute, Inc.). Mixed models were estimated with the REML method. For all tests, I checked for equal standard deviations among treatment groups and/or examined residual plots to make sure that the distribution of errors was not skewed. I report means and least squares (LS) means with 95% confidence intervals as: estimated mean (lower limit, upper limit).

RESULTS

Short-term responses

As expected, bumblebees probed many more flowers per inflorescence at station 1 during nectar-enriched trials (LSmean = 11.56 flowers (10.68, 12.44)) than during control trials (8.18 flowers (7.30, 9.06)). Relative to control trials, bees also probed a larger number of flowers per inflorescence, on average, at stations 2 and 3 after first visiting the nectar-enriched station (Fig. 2; $F_{1,15} = 6.71$, $P = 0.021$). Overall, the mean number of flowers probed per inflorescence was greater at station 2 ($F_{1,30} = 12.4$, $P = 0.0014$), but there was no interaction between nectar environment and station number ($F_{1,30} = 0.05$, $P = 0.83$).

During nectar-enriched trials, bumblebees also visited a greater number of inflorescences per trial at stations 2 and 3 relative to control trials (estimated difference: 0.43 inflorescences (0.05, 0.81); paired t-test, $t_{15} = 2.42$, $P = 0.029$).

Long-term responses

The 'over-visitation' effect observed in my array experiment diminished slowly over time (Figure 3), presumably as individual bees returned to the focal pair of inflorescences. The estimated mean number of flowers probed per visit to control inflorescences was significantly greater during E→C, relative to C→E transitions only in the first two hours of observation (paired t-test, $t_7 = 4.13$ and 2.44, $P = 0.0044$ and 0.045 for hours 1 and 2, respectively). There was, however, no detectable difference in the mean number of flowers probed per visit to C and E inflorescences during the third ($t_7 = -1.26$, $P = 0.25$) or fourth ($t_7 = -0.56$, $P = 0.59$) hour of repeated nectar additions. This temporal change in average bumblebee behaviour was apparently consistent among individual bees. The mean standard deviation in number of flowers probed/visit at the control inflorescence during E→C transitions did not differ among hours ($F_{3,28} = 0.83$, $P = 0.49$).

On average, bumblebees visited control inflorescences at a greater frequency than the nectar-enriched inflorescence (paired t-test, $t_7 = 8.55$, $P < 0.0001$). Over the four-hour period, I could detect no change in the frequency of bumblebee visits to the two focal inflorescences. The difference between E and C visits did not vary, on average, among hours ($F_{3,28} = 0.40$; $P = 0.76$). Similarly, the difference in the number of E→C transitions, relative to C→E, did not differ among hours ($F_{3,28} = 0.45$; $P = 0.72$).

DISCUSSION

Chamerion angustifolium is often recognized as a prolific nectar producer, relative to other species that are commonly visited by nectar-foraging bumblebees (Heinrich, 1979). Investment to nectar production in *C. angustifolium* is therefore a good candidate for a character that has been exaggerated through success in social competition (Fig.1). To determine the efficacy of this model, one must first establish the extent to which pollinator service depends on relative reward quality. My experiments here illustrate pollinator responses that depend on relative reward value, but do not offer evidence of social competition among locally competing plants. Instead, my results highlight a limitation of small-scale experiments used to investigate social competition in natural populations. I discuss these caveats further and then consider an important link between social competition and sexual selection in flowering plants.

Mechanisms of social competition

Number of flowers probed per visit

Foragers may leave a resource patch when their energy intake rate falls below the remembered intake rate of other patches in the population (see Ollason, 1980). In this regard, bumblebees apparently have a short memory; the patch departure threshold of nectar-foraging bees often depends on only the last few inflorescences visited (Cibula and Zimmerman, 1987). Thus, it is reasonable to expect recently-rewarded bumblebees to probe fewer flowers at non-enriched plants than they would if not initially rewarded.

After visiting nectar-enriched inflorescences in my experimental arrays, however, bumblebees' immediate response was to *increase* the number of flowers probed on subsequently visited inflorescences. This observation is consistent with the behaviour of bumblebees on *Aralia hispida* that had first visited a nectar-enriched inflorescence, which Thomson et al. (1982) interpreted as a form of area-restricted searching. In my study, the 'over-visitation' was maintained with continued sampling, as bumblebees moved through the linear array (i.e. no nectar environment-by-station number interaction). Furthermore, bumblebees in the nectar-enriched environment visited a greater number of inflorescences within the array, relative to control trials. Overall, these results are contrary to the predicted effects of social competition acting via manipulation of pollinator searching behaviour. Bees did not reject relatively unrewarding inflorescences in a nectar-enriched environment, nor did they quickly pass through patches of relatively unrewarding inflorescences.

Moreover, I found that the 'over-visitation' of non-enriched inflorescences (after first visiting an enriched inflorescence) was ephemeral. Presumably, individual bumblebees returned to the focal patch and learned that only one inflorescence of the pair was nectar-enriched. With time, the number of flowers probed on the non-enriched inflorescence apparently did not depend simply on the remembered intake rate at recently visited inflorescences, but also on bumblebees' long-term memory for the location of the enriched inflorescence. In fact, by the end of the *long-term responses* experiment, the number of flowers probed at a non-enriched inflorescence was independent of the rewards offered by its neighbour.

It seems likely that the direction of the pollinator response to a nectar-enriched environment depends on the frequency of the enriched phenotype in the population (or the spatial scale of nectar manipulations). When the nectar-rich phenotype is rare, pollinators initially use an area-restricted searching behaviour, but as the nectar-rich phenotype increases in frequency, I expect that pollinators may reject relatively less rewarding plants (given that many more rewarding options are available). Thus, to evaluate the significance of social competition acting via pollinator searching behaviour, an experiment of much larger scale than the current study may be useful. Such an experiment would require large-scale manipulations of nectar production (i.e. by watering and/or fertilizing groups of plants).

Frequency of visits to a plant

Bumblebees probed many flowers, and potentially removed large amounts of pollen, per visit to nectar-enriched inflorescences (see Fig. 3). Because of diminishing returns for increased pollen removal (reviewed by Harder et al., 2001), it is unlikely that a relatively rewarding plant could accrue increased absolute fitness unless pollinators also increase their *rate* of visitation to that plant. Over the duration of my repeated nectar additions, I could detect no change in the rate of visitation to either the control or nectar-enriched inflorescences. Unfortunately, my experimental manipulations may not have lasted long enough to detect such an effect. Given time, bumblebees may eventually learn the location of, and preferentially visit nectar-enriched plants (Thomson, 1988), or patches of plants with high rates of nectar production (Klinkhamer et al., 2001).

The ESS investment to nectar rewards should depend on pollinators' ability to discriminate among plants of varying quality. When pollinator discrimination is poor, the ESS investment to nectar production should be less than if pollinators precisely favour plants with relatively high rates of production. In mixed patches of plants with both high and low rates of nectar production, the frequency of bumblebee visits to a plant is not always positively related to the nectar production rate (Klinkhamer et al., 2001, Klinkhamer and van der Lugt, 2004). Thus, in some cases, relatively poor nectar producers may benefit from the presence of more rewarding neighbours. Future studies must focus not only on whether pollinators preferentially visit relatively rewarding plants, but whether increased visitation to rewarding plants comes at the expense of less rewarding competitors. That is, it is necessary to understand the extent to which pollinator service can be considered a finite, limited resource.

Implications for sexual selection in plants

Angiosperm characters that reward (or signal reward to) their animal pollinators are often interpreted as products of sexual selection (reviewed by Andersson, 1994, Skogsmyr and Lankinen, 2002). Sexual selection in this sense is defined rather broadly, however, as selection that arises from differential mating success (e.g. Arnold, 1994, Willson, 1990). In contrast, the concept of sexual selection in animals typically emphasizes the special problem of extravagance in mating characters (e.g. Zahavi, 1991).

Current evidence from animal-pollinated plants is insufficient to understand the adaptive significance of seemingly extravagant floral phenotypes. To my knowledge, no study has explicitly addressed whether a particular character provides an absolute reproductive benefit (i.e. a benefit that is independent of the phenotypes of competitors), or whether it also functions to maintain a plant's relative attractiveness to pollinators (as in social competition). Grant (1995) implicitly used a similar distinction between absolute and relative benefits to argue that secondary sexual characters are currently unknown in plants.

Success in social competition for access to a critical resource (including mates) is the common selection pressure that favours the evolution and maintenance of extravagance (West-Eberhard, 1983). Thus, I suggest that floral phenotypes that function in social competition for pollinator service should be considered analogous to the secondary sexual characters of animals. The prospect of uncovering true secondary sexual characters in flowering plants is an appealing incentive to understand the pollinator attraction game.

LITERATURE CITED

Abrams, P.A. and Matsuda, H. 1994. The evolution of traits that determine ability in competitive contests. *Evolutionary Ecology*, **8**: 667-686.

Andersson, M. 1994. *Sexual Selection.* Princeton, NJ: Princeton University Press.

Arnold, S.J. 1994. Is there a unifying concept of sexual selection that applies to both plants and animals? *American Naturalist*, **144**: S1-S12.

Charlesworth, D. and Charlesworth, B. 1987. The effect of investment in attractive structures on allocation to male and female function in plants. *Evolution*, **41**: 948-968.

Charnov, E.L. 1982. *The Theory of Sex Allocation.* Princeton, NJ: Princeton University Press.

Charnov, E.L. and Bull, J.J. 1986. Sex allocation, pollinator attraction and fruit dispersal in cosexual plants. *Journal of Theoretical Biology*, **118**: 321-325.

Cibula, D.A. and Zimmerman, M. 1987. Bumblebee foraging behavior: changes in departure decisions as a function of experimental nectar manipulations. *American Midland Naturalist*, **117**: 386-394.

Grant, V. 1995. Sexual selection in plants: Pros and cons. *Proceedings of the National Academy of Sciences, USA*, **92**: 1247-1250.

Harder, L.D., Williams, N.M., Jordan, C.Y. and Nelson, W.A. 2001. The effects of floral design and display on pollinator economics and pollen dispersal. In *Cognitive Ecology of Pollination* (Chittka, L. and Thomson, J.D., eds), pp. 297-317. Cambridge University Press.

Heinrich, B. 1979. *Bumblebee economics.* Harvard University Press.

Klinkhamer, P.G.L. and deJong, T.J. 1993. Attractiveness to pollinators: a plant's dilemma. *Oikos*, **66**: 180-184.

Klinkhamer, P.G.L., deJong, T.J. and Metz, A.J. 1994. Why plants can be too attractive--A discussion of measures to estimate male fitness. *Journal of Ecology*, **82**: 191-194.

Klinkhamer, P.G.L., de Jong, T.J. and Linnebank, L.A. 2001. Small-scale spatial patterns determine ecological relationships: An experimental example using nectar production rates. *Ecology Letters*, **4**: 559-567.

Klinkhamer, P.G.L. and van der Lugt, P.-P. 2004. Pollinator service only depends on nectar production rates in sparse populations. *Oecologia*, **140**: 491-494.

Maynard Smith, J. 1982. *Evolution and the Theory of Games.* Cambridge University Press.

Maynard Smith, J. and Brown, R.L. 1986. Competition and body size. *Theoretical Population Biology*, **30**: 166-179.

Morgan, M.T. 1992. Attractive structures and the stability of hermaphroditic sex expression in flowering plants. *Evolution*, **46**: 1199-1213.

Ollason, J.G. 1980. Learning to forage--optimally? *Theoretical Population Biology*, **18**: 44-56.

Parker, G.A. 1983. Arms races in evolution--An ESS to the opponent-independent costs game. *Journal of Theoretical Biology*, **101**: 619-648.

Parker, G.A. and Maynard Smith, J. 1990. Optimality theory in evolutionary biology. *Nature*, **348**: 27-33.

Pyke, G.H. 1980. Optimal foraging in nectar-feeding animals and coevolution with their plants. In *Foraging behaviour: Ecological, Ethological and Psychological Approaches* (Kamil, A.C. and Sargent, T.D., eds), pp. New York: Garland.

Reeve, H.K. and Sherman, P.W. 1993. Adaptation and the goals of evolutionary research. *Quarterly Review of Biology*, **68**: 1-32.

Sakai, S. 1993. A model for nectar secretion in animal-pollinated plants. *Evolutionary Ecology*, **7**: 394-400.

Schmid-Hempel, P. and Speiser, B. 1988. Effects of inflorescence size on pollination in *Epilobium angustifolium. Oikos*, **53**: 98-104.

Skogsmyr, I. and Lankinen, A. 2002. Sexual selection: an evolutionary force in plants? *Biological Review*, **77**: 537-562.

Thakar, J.D., Kunte, K., Chauhan, A.K., Watve, A.V. and Watve, M.G. 2003. Nectarless flowers: ecological correlates and evolutionary stability. *Oecologia*, **136**: 565-570.

Thomson, J.D., Maddison, W.P. and Plowright, R.C. 1982. Behavior of bumble bee pollinators of *Aralia hispida* Vent. (Araliaceae). *Oecologia*, **54**: 326-336.

Thomson, J.D. 1988. Effects of variation in inflorescence size and floral rewards on the visitation rates of traplining pollinators of *Aralia hispida. Evolutionary Ecology*, **2**: 65-76.

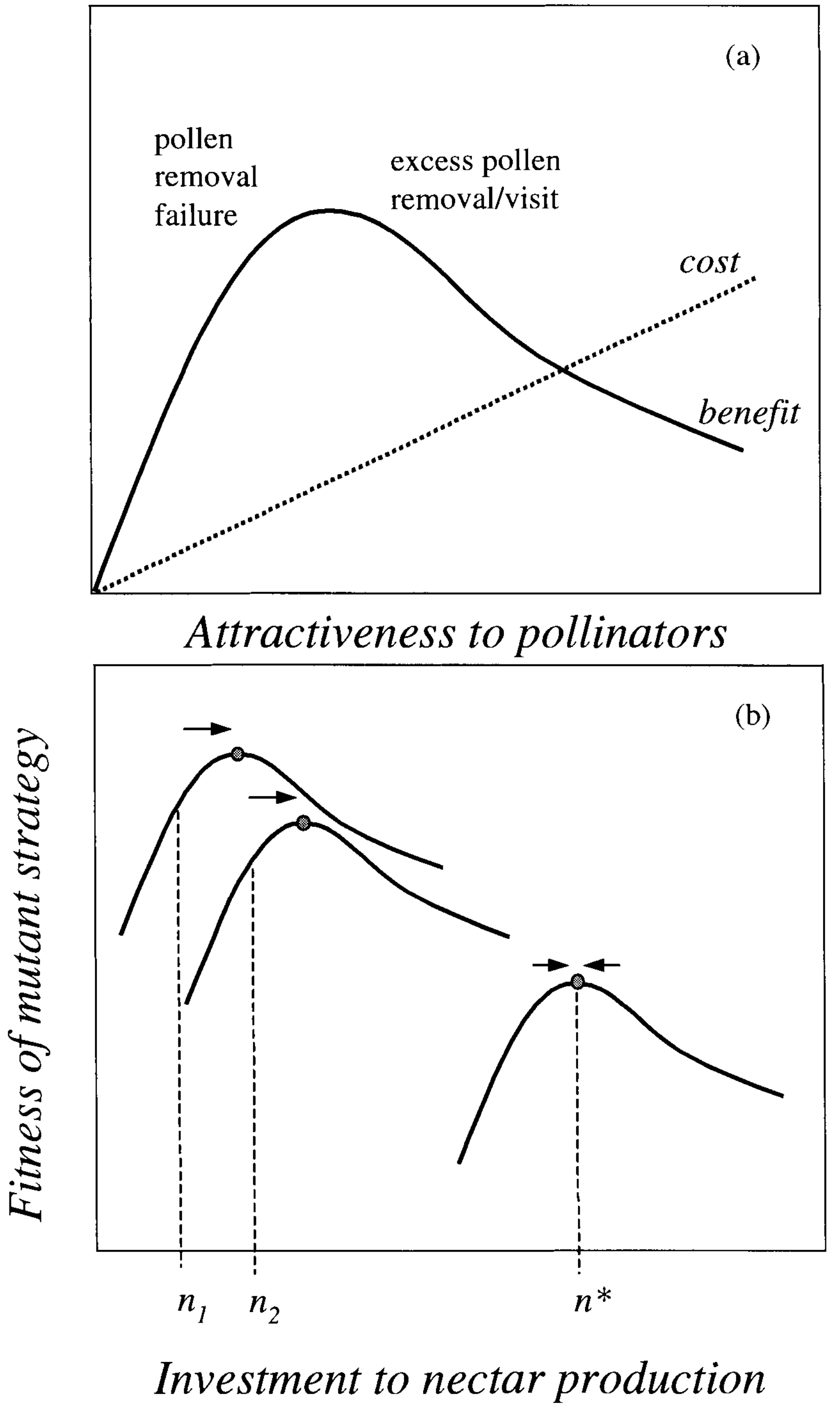
West-Eberhard, M.J. 1983. Sexual selection, social competition, and speciation. *The Quarterly Review of Biology*, **58**: 155-183.

Willson, M.F. 1990. Sexual selection in animals and plants. *Trends in Ecology and Evolution*, **5**: 210-214.

Zahavi, A. 1991. On the definition of sexual selection, Fisher's model and the evolution of waste and of signals in general. *Animal Behaviour*, **42**: 501-503.

FIGURES

Figure 2-1 (a). Plant fitness is determined by the difference between the benefit of attracting pollinators and the cost of producing rewards such as nectar. The cost of nectar production is assumed to increase linearly, but the benefit of attracting pollinators increases with strong diminishing returns. Absolute fitness increases with further attraction of pollinators only if the pollen removal of each individual visitor can be restricted. (b). Evolution of nectar production under social competition for pollinator service. The benefit of a particular investment to nectar production (i.e. attractiveness to pollinators) depends on a comparison with the mean investment of the population (dashed lines). The cost of nectar production is incurred regardless of competitors' strategies. There is an initially positive fitness benefit for being more rewarding than the mean strategy because fitness is limited by access to pollinators. For example, when the population mean strategy is n_1 , the best strategy to adopt is n_2. But as the population mean approaches n_2 , the optimum investment is still higher, because it takes an even larger expenditure to maintain attractiveness to pollinators. Population mean fitness declines because plants pay a progressively higher cost to maintain the same level of benefit. No mutant strategy has higher fitness than the ESS (n^*), where the cost of further investment is greater than the potential disadvantage of competing against a slightly more attractive strategy. (Adapted with permission from Parker and Maynard Smith, 1990).


(a)
pollen removal failure
excess pollen removal/visit
cost
benefit
Attractiveness to pollinators
(b)
Fitness of mutant strategy
n_1
n_2
n^*
Investment to nectar production

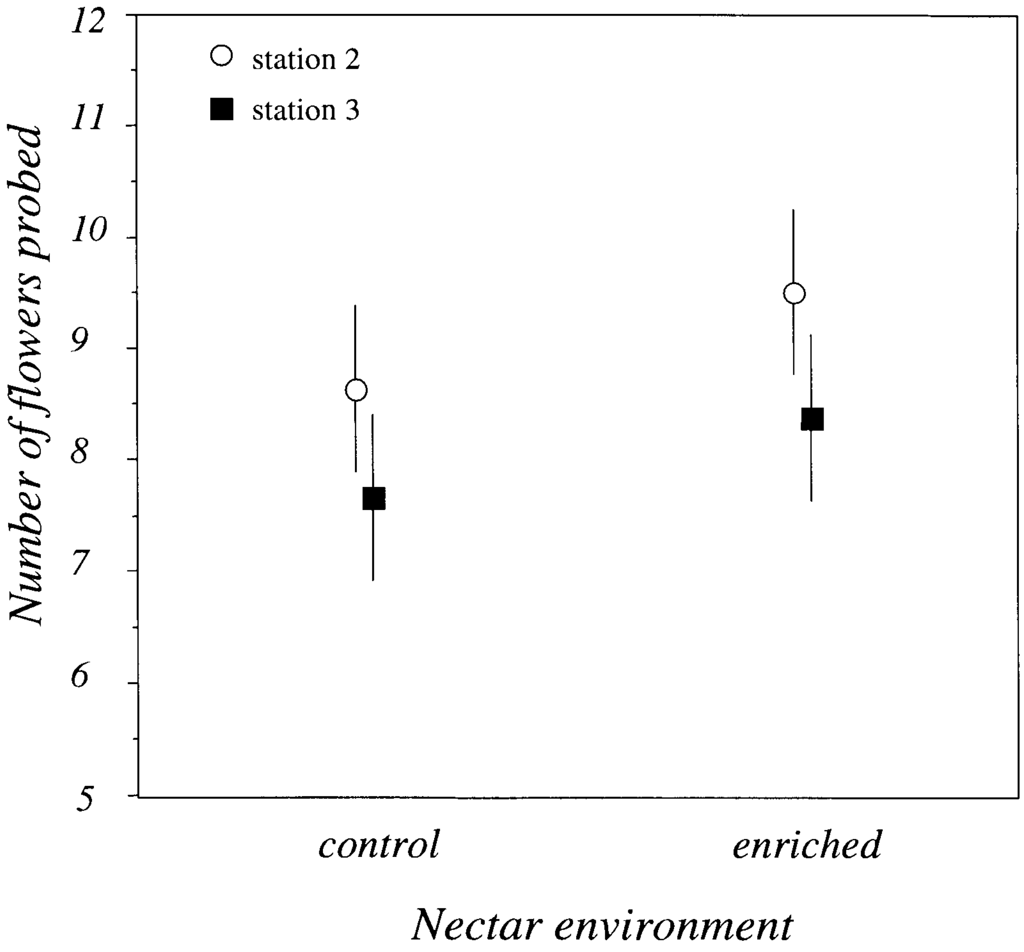


Figure 2-2 Least squares mean (± 95% C.I.) number of flowers probed per (non-enriched) inflorescence at stations 2 and 3, depending on the nectar quality at station 1. In all cases, bumblebees visited stations 1 through 3 in sequence. Overall effect of station (estimated difference): 1.04 flowers (0.44, 1.64); overall effect of nectar environment: 0.79 flowers (0.14, 1.44).

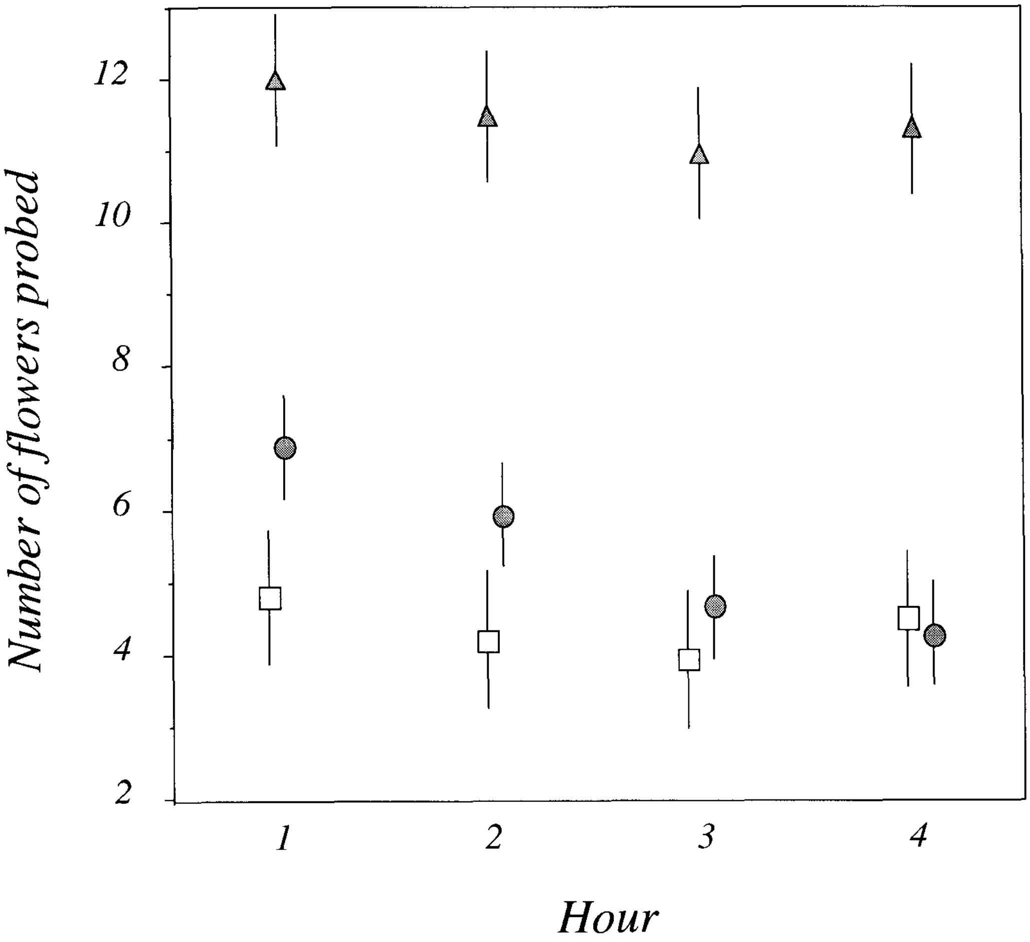


Figure 2-3 Least squares mean (± 95% C.I.) number of flowers probed by bumblebees on nectar-enriched inflorescences (Δ), and on a neighbouring control inflorescence, before (□) or after (O) visiting the nectar-enriched neighbour in sequence. Nectar was replenished over a 4 hour period; means are given for each hour. After being initially rewarded (T-->C), bumblebees probed significantly more flowers on the control inflorescence, relative to C-->T transitions, but only during the first two hours of repeated nectar additions.

CHAPTER 3 GENDER AND REWARDS VARY WITH COMPETITIVE ABILITY IN AN ANIMAL-POLLINATED PLANT

ABSTRACT

The largest plants in a population typically display the most flowers and receive the most pollinator visits. Male fitness should increase with flower number, but at a decelerating rate if pollen removal during individual visits also increases with flower number. I investigate size-dependent nectar production and gender allocation tactics of *Chamerion angustifolium* (Onagraceae). Flowers are borne on vertical inflorescences, with male-phase flowers positioned above female-phase flowers. Small inflorescences distributed nectar nearly evenly among female- and male-phase flowers, whereas larger inflorescences allocated extra nectar to female-phase flowers. In experimental inflorescences, I distributed the same volume of nectar to mimic the 'large' (uneven) and 'small' (even) allocation types. Nectar-foraging bumblebees probed a mean of 3.2 fewer male-phase flowers per visit to the 'large', relative to 'small' type, as expected if the nectar distribution of large displays functions to limit pollen removal. On a per gram basis, the largest ramets in the population produced ca. 1/3 the number of flowers but a roughly equal number of seeds compared to smaller ramets. Based on similar seed: flower ratios in other systems, some authors have concluded that large plants emphasize female reproduction. However, these investigators ignored the opportunity for increased

maleness through extra reward production, as I suggest for the largest *C. angustifolium* ramets.

INTRODUCTION

Individual size differences within a population will often reflect variation in competitive ability and current fitness attainment ("winnings", Williams, 1992). A positive relation between size and phenotypic fitness may be especially evident among flowering plants. Relative to smaller individuals, large plants can display a greater number of rewarding flowers at once (e.g. Chaplin and Walker, 1982, Worley et al., 2000) and mature a greater number of seeds (e.g. Dudash, 1991, Solbrig and Solbrig, 1984). Large floral displays should be particularly beneficial for outcrossing, animal-pollinated plants because pollinators visit large displays more frequently than smaller conspecifics (reviewed by Ohashi and Yahara, 2001), and a high visitation rate (herein 'attractiveness') is critical for the male function of inflorescences (i.e. pollen export, see Harder et al., 2001).

Although male success should generally increase with attractiveness to pollinators, there is a sense in which plants can be too attractive. The fraction of removed pollen that remains available for export should decline as the amount of pollen removed per visit increases (e.g. Klinkhamer et al., 1994). These diminishing returns exist if, for example, pollinators become saturated with pollen or if pollen grooming behaviour increases with removal (reviewed by Harder et al., 2001). And because pollinators probe more flowers per visit to large displays (Ohashi and Yahara, 2001), the potential costs of excess pollen removal are especially apparent for the most attractive plants in a population. Here, I explore tactics that plants might use to maximize fitness, depending on ability to attract pollinators.

Size-dependent nectar production

The largest floral displays in a population could maximize their advantage if many different pollinators were to remove only small amounts of pollen or probe only a few pollen-bearing flowers at a time (Harder and Thomson, 1989, Iwasa et al., 1995). Plants with less attractive displays must balance the benefit of removal restriction with the even larger cost associated with pollen removal failure; hence, pollen export from smaller displays is maximized with fewer restrictions on pollen removal (Harder et al., 2001). One mechanism by which the optimal pollen dispensing schedule might be adjusted to attractiveness is by varying the distribution of floral nectar production rates (NPRs) within inflorescences (Biernaskie and Cartar, 2004). In nature, the flowers of vertical inflorescences are often protandrous and open so that the youngest (male-phase) flowers are positioned above younger (female-phase) flowers. Many displays of this type produce nectar in a gradient that decreases from bottom to top; accordingly, nectarivores approach the lowermost flowers, move upwards, and leave when energy intake declines to a threshold rate (e.g. Best and Bierzychudek, 1982, Hodges, 1985, Pyke, 1978). Plants could mediate the number of pollen-bearing flowers probed per visit by adjusting the rate at which energy gains reach the departure threshold (Ohashi and Yahara, 2001). If plants can adjust the nectar gradient conditionally on display size, then I expect large floral displays to produce a stronger nectar gradient than smaller, competing conspecifics. This should promote earlier departure, limit pollen removal, and minimize the diminishing returns associated with attractiveness.

Size-dependent gender allocation

The strength of diminishing returns to male fitness is a critical determinant of size-dependent gender allocation. Given a difference in the shape of male and female fitness gain curves (fitness with respect to investment to each gender function), simultaneous hermaphrodites that differ in relative size should also differ in the proportion of energy that is allocated to each gender function (Charnov, 1982, Lloyd and Bawa, 1984). For example, if both male and female gain curves show diminishing returns (both exponents < 1) but the male gain curve decelerates faster, then an emphasis on female function is evolutionarily stable for relatively large individuals. With the same ratio of exponents, small individuals gain a greater male fitness return per unit of investment because their total investment into reproduction is small (the "budget effect" of Klinkhamer et al., 1997). Relatively small plants should therefore emphasize male function.

Evidence from natural populations suggests that relatively large plants do in fact allocate disproportionately to female function and vice versa (e.g. Klinkhamer et al. 1997 and refs. therein; Sarkissian et al. 2001). This pattern implies that the diminishing returns to male function exceed any potential diminishing benefits for increased female allocation (e.g. local resource competition, Lloyd and Bawa, 1984). If, however, individuals have special mechanisms to minimize the potential costs of attraction (last section), then allocation to male function may increase nearly proportionately with plant size (see Ishii, 2004).

Objectives

I investigate size-dependent reproductive allocation in *Chamerion angustifolium* (Onagraceae)—a simultaneous hermaphrodite with vertical inflorescences and structured dichogamy. I determine:

- ☐ whether the strength of the nectar gradient varies positively with floral display size
- ☐ the effect of alternative nectar gradients on bumblebees': (*i*) arrival position and inter-floral movements, (*ii*) departure decisions, and (*iii*) pollen removal
- ☐ the pattern of size-dependent gender allocation, measured as flower production (an upper estimate of male investment; after Klinkhamer and deJong 1997) versus seed production (female investment).

METHODS

Chamerion (=Epilobium) angustifolium (L.) Holub is a perennial wildflower, commonly found in disturbed areas of North America and Europe. Newly opened flowers gradually expose pollen from eight anthers that dehisce sequentially. The male-phase lasts about 2.5 days, at which time the stigma becomes receptive. Flowers are female-phase for an additional 1.5 days before wilting (Sargent and Roitberg 2000). Floral displays at my site ranged from 6-27 flowers. The most common pollinators observed were nectar-collecting worker bumblebees (*Bombus* spp.; mainly *B. flavifrons* and *B. bifarius*), which visited the displays frequently (~ once every 3 minutes).

In the summers of 2002 and 2003, I studied *C. angustifolium* on Silver Star Mountain, near Vernon, British Columbia, Canada (50.3° N, 119.3° W), where large populations are found along roadsides and in the clearings of Silver Star Resort. At my study sites, ramets flower for ~ 4-6 weeks in July and August, after which dehiscent fruits release hundreds of tufted seeds.

Size-dependent nectar production

a. Field observations

Ramets for nectar measurements were haphazardly sampled to span the range of observed floral display sizes. I sampled ramets from throughout 4 large focal patches, where the display size distributions were not obviously different. At each observation period, at least two ramets from each size category—small (< 9 flowers), medium (~12 flowers), and large (>15 flowers)—were selected. I chose plants that were at peak flowering stage (those with flower buds and developing fruits) and that were not obviously part of the same genet. Flowers were emptied of any standing nectar by gentle probing with 2 μl microcapillary tubes. Whole inflorescences were then covered with bridal veil bags to exclude pollinators for 4 sunny daylight hours.

Bagged ramets were clipped off at the base and nectar production was measured within 1 hour of collection. For each ramet, I recorded height, display size (number of open flowers and their gender phase), and the mean 4-hour nectar production of three randomly selected male- and three randomly selected female-phase flowers. I did not measure intermediate flowers (no pollen remaining and stigma not yet receptive) or newly opened flowers without exposed pollen. I extracted nectar with 2 ul microcapillary tubes and measured the sugar concentration (mg solute/ml solution) of each sample with a hand-held refractometer (ATAGO, Japan); nectar production was later converted to Joules (J) of energy.

Analysis. I tested whether NPR varied with the main effects of 'gender phase', 'display size', and their interaction (SAS, PROC MIXED). 'Ramet' was included as a subject variable to account for the two (non-independent) nectar production measures (mean male-phase, mean female-phase production) within each ramet.

b. Nectar manipulations and bumblebee behaviour

(i) Gradient direction. Bumblebee arrival positions and inter-floral movements were compared on opposite nectar gradients. In large patches, I selected a focal inflorescence and standardized the display size to 10 flowers. The inflorescence was randomly assigned to a 'reinforced' (2 µl of 42% sucrose solution ('nectar') added to each of four lowermost flowers) or 'reversed' treatment level (2 µl to each of four uppermost flowers). I emptied any standing nectar in manipulated flowers (rarely present) and replenished nectar levels after each bumblebee visit for three consecutive hours of observations at each ramet (for a total of 15 ramets per treatment level). During observation periods, I recorded the vertical position of the first three flowers probed on the focal inflorescence (where '1' is the lowermost flower) and the relative position of the first flower probed after departure from the focal inflorescence (probed flower's position/ total number of open flowers).

Analysis. I calculated mean flower positions probed per foraging bout for each ramet (n = 30). To ensure that the data reflected visits in which bees experienced a sufficiently large treatment, I excluded those bouts on 'reinforced' gradients in which less than two enriched flowers were probed (17 bouts). Similarly, before analysing the post-departure data, I excluded those bouts on 'reversed' gradients in which less than two enriched flowers were probed (73 of 431 bouts). Treatment level means were compared with a single factor analysis of variance (ANOVA).

(ii) Gradient strength. Bumblebee bout lengths (number of flowers probed before departure) were compared on inflorescences to which the same volume of nectar was added in alternative distributions. All floral displays were first trimmed to 11 flowers (three female phase flowers, eight male-phase). Two treatment levels—a 'strong' gradient (0.5 μl of 42% sucrose solution to each of three lowermost flowers) and a 'weak' gradient (0.25 μl added to each of six lowermost flowers)—were assigned in random order to each of 17 ramets. I recorded the total number of flowers probed and the number of those that were male-phase. Trials lasted for at least five bumblebee visits (with nectar levels replenished between visits) but were extended until at least two visitors probed all enriched flowers in a single bout.

Analysis. I calculated the mean number of flowers probed per foraging bout for each ramet-treatment level combination (n = 34), using only those visits in which all enriched flowers were probed. (Results did not differ when all visits were included). Treatment level means were compared with ANOVA, using 'ramet' as a (random) blocking factor, within which each treatment level appeared once.

(iii) Pollen removal. I compared the handling times and pollen removal from individual flowers that were or were not enriched with nectar. On each of 15 different ramets, two flowers with undehisced anthers were enclosed with small bridal veil bags to exclude visitors. When anthers had dehisced, I carefully removed the bags and emptied flowers of all standing nectar without contacting the anthers. Two anthers with fully exposed pollen were chosen from each flower; all others were clipped off. One of the

focal flowers on each ramet was then randomly selected for a 2 μl (40% sucrose) nectar addition; the second flower served as a non-enriched control. Trials lasted for two foraging bouts by (presumably) two different bees that probed both the enriched and control flower. As above, I checked for nectar removal and replenished the enriched flower after the first visit. For each visit, I recorded the handling time of focal flowers (time between landing and leaving a flower) with a stopwatch. Following the second bout, the anthers of each flower were collected in separate vials. I estimated the number of remaining pollen grains per flower by suspending pollen in 25μl of (3: 1) lactic acid: glycerine solution, and counting two 5 ul samples on a haemocytometer under compound microscope.

Analysis. I estimated the mean number of pollen grains remaining for each ramet-treatment level combination (n = 30). Treatment level means were compared with ANOVA, using 'ramet' as a (random) blocking factor, as above.

Size-dependent gender allocation

I erected a 4 m x 2 m sampling frame in each of two dense patches of *C. angustifolium* at Silver Star Resort. Three random points were selected along the length of each frame, where transects were initiated to span the 2 m width. At 50 cm intervals along each transect, I selected the largest and smallest ramets (based on stem thickness) within at most 30 cm of the selected point and perpendicular to the transect. Thus, a total of 60 ramets were selected by 6 repeated systematic samples. My selection criteria were designed to include a large range of ramet sizes in each transect sample.

Five undehisced fruits per ramet were collected over two dates in late September, 2003. On the first date, 2 or 3 fruits were randomly selected from those mature enough to collect, depending on whether less than or more than half, respectively, of the ramet's fruits were available. The remaining fruits were collected ~2 weeks later by a random selection of the fruits that were unavailable on the first collection date. Above-ground biomass of each ramet was also collected at this time.

Seed set per fruit was determined by counting the number of inflated ovules (see Wiens et al. 1987) under stereoscope from those fruits collected at positions 1, 3, and 5 of my random sample from each ramet. For each ramet, I recorded the height, total fruit production and total flower production (= # fruits + # undeveloped ovaries). Total seed production was estimated as average inflated ovules/fruit * total fruits. Vegetative biomass was dried at 70°C for 48 hours and weighed to the nearest hundredth of a gram.

Analysis. I used a simple allometric model to examine the relation between ramet biomass (x) and seed, fruit, or flower production (y's) (after Klinkhamer and deJong 1997):

$$y = a\,x^b \qquad or \qquad \log y = a + b \log x$$

Linear regression of log-transformed data gives an estimate of the slope b, which indicates a more than ($b > 1$) or less than ($b < 1$) proportional increase in allocation with ramet biomass.

In accordance with repeated systematic sampling methods (Levy and Lemeshow, 1999), an estimated slope was determined for each transect and then used to calculate the overall mean estimate, b^* (the overall effect size, from n = 6) and its 95% confidence interval. The overall mean estimate was also used for hypothesis testing (e.g. H_0: $b^* = 1$). I had little power to detect differences between the two patches; hence, this effect was ignored in my analyses. Where pooled data are presented, I use leverage plots (JMP Start

Statistics, SAS Institute, Inc.) that account for variation in the data due to 'transect' (random factor); reported statistics, however, are calculated from the mean of each transect's slope, as above.

Throughout the chapter, mixed models were estimated with the REML method. For all tests, I checked for equal standard deviations among treatment groups and/or examined residual plots to check that the distribution of errors was not skewed. Below, means or least squares (LS) means are given with 95% confidence intervals as: estimated mean (lower limit, upper limit). All differences between means were estimated with Tukey-Kramer independent contrasts.

RESULTS

Correlates of resource state

Above ground biomass was my measure of resource state. In the sample of plants collected for biomass measures, ramet height correlated positively with mass (Pearson correlation coefficient, $r = 0.90$, $P < 0.0001$, $n = 58$). Thus, height may be considered a surrogate for resource state. In the field, the height of ramets selected for nectar measurements correlated positively with observed display size ($r = 0.71$, $P < 0.0001$, $n = 45$), and (marginally) with mean 4-hour nectar production (J) per flower ($r = 0.25$, $P = 0.098$, $n = 45$).

Size-dependent nectar production

a. Field observations

The average difference between the NPRs of female- and male-phase flowers increased with floral display size. I detected a display size-by-gender phase interaction ($F_{1,43}$ = 9.12, $P = 0.0042$) because large displays allocated differentially to nectar production in female- relative to male-phase flowers (Fig. 1). In my study populations, the proportion of open flowers that were in female-phase decreased only slightly with increased floral display size ($r = -0.28$, $P = 0.064$, $n = 45$).

Averaged across all display sizes, male-phase flowers produced less nectar than female flowers (mean difference: 5.3 J (4.3, 6.3)); in addition, male-phase flowers take longer to handle (see Appendix I). In a small experiment, wild bumblebees spent a mean of 4.6 sec (4.3, 5.0) on male-phase flowers that contained 1 µl of nectar compared to 2.8 sec (2.5, 3.1) on female-phase flowers with the same volume added (n = 36 bees/treatment level).

b. Nectar manipulations and bumblebee behaviour

(i) Gradient direction. I detected no difference in the mean position of the first flower probed on 'reinforced' and 'reversed' nectar gradients ($F_{1,28} = 0.28$, $P = 0.60$; Fig. 2), as expected if bees do not detect nectar remotely. However, bumblebees tended to move shorter distances as they moved upwards on the reinforced gradients; the mean position of the first three flowers probed in sequence was, on average, lower on reinforced gradients ($F_{1,28} = 4.94$, $P = 0.0345$; Fig. 2). After leaving a reinforced gradient, the mean vertical position of the next flower probed in the patch (flower position/display

size) was 0.21 (0.17, 0.25); after leaving a reversed gradient, the mean position probed was significantly higher (0.28 (0.24, 0.32); $F_{1,28} = 6.51$, $P = 0.0165$).

(ii) Gradient strength. Bumblebees probed a larger number of flowers in sequence before departure of 'weak' gradients compared to 'strong' ones ($F_{1,16} = 54.9$, $P < 0.0001$). The difference in number of flowers probed can be accounted for by an increased number of flowers probed on upper, male-phase flowers (Fig. 3).

(iii) Pollen removal. Individual bumblebees spent significantly more time handling nectar enriched male-phase flowers relative to control male-phase flowers on the same inflorescence (mean difference = 6.12 seconds (5.01, 7.22), n= 28 bees). The amount of pollen remaining in nectar enriched flowers was significantly less than that remaining in control flowers ($F_{1,14} = 31.3$, $P < 0.0001$); the estimated mean difference in pollen remaining per flower pair was 288.7 grains (178.0, 399.5).

Size-dependent gender allocation

Gender allocation, measured by total flower and seed production, varied considerably with ramet biomass. The largest ramets in the population produced disproportionately fewer flowers and fewer fruits than smaller ramets; the estimated slope b^* was less than unity in both cases (Table 1; one sample t-test, $t_5 = -7.5$ and -7.8, respectively; both $P < 0.001$). The relation between ramet biomass and total seed production/ramet was, however, not detectably different from unity (Table 1; $t_5 = -0.75$, $P = 0.49$). Thus, larger plants produced, on average, fewer flowers per gram of biomass, but a greater number of seeds per flower (Fig. 4).

The higher per-flower seed set in large ramets, relative to smaller ramets, may reflect greater pollination success (i.e. the large number of non-inflated in small ovules in small ramets may have been unfertilized). I randomly selected 30 of the collected ramets from which to determine the inflated: non-inflated ovule ratio, using the remaining fruits (positions 2 and 4) from each ramet. (Data were treated as a simple random sample). There was no detectable relation between ramet biomass and the mean inflated: non-inflated ratio per flower (linear regression, $F_{1,28} = 1.3$, $P = 0.27$), however, because total ovule number/flower (inflated + non-inflated) increased with ramet size ($F_{1,28} = 37.7$, $P < 0.0001$). Hence, there was a negative relation between the total number of non-inflated (potentially aborted) ovules/ g biomass and ramet biomass ($F_{1,56} = 125.4$, $P < 0.0001$), because small ramets produced more flowers/ g biomass and vice versa.

DISCUSSION

Within *Chamerion angustifolium* populations, the largest ramets should experience the highest reproductive potential through both male and female function. Large ramets produce the largest floral displays, most nectar, and most seeds (this study), and pollinators visit large displays at highest frequency (JMB, unpub. data, Sargent and Roitberg, 2000, Schmid-Hempel and Speiser, 1988). Given that the success of alternative allocation tactics depends on size, selection should favour allocation rules that specify the optimal tactic in relation to size (or quality of environment; e.g. Zhang and Jiang, 2002). I observed variation in *C. angustifolium* nectar production and gender allocation phenotypes that may be components of adaptive, size-dependent allocation rules.

The nectar distribution as a pollen dispensing mechanism

The distribution of nectar production in vertical inflorescences with structured dichogamy— typically decreasing from bottom to top—has long been presumed to increase the efficiency of cross-pollination (e.g. Best and Bierzychudek, 1982, Pyke, 1978). Specifically, because pollinators tend to move upwards on inflorescences regardless of rewards (this study, Corbet et al., 1981, Orth and Waddington, 1997, Waddington and Heinrich, 1979) high nectar production rates in lower flowers may ensure that pollinators enter low enough on the display to import foreign pollen to female-phase flowers. My results here also demonstrate that when foraging on a reinforced (relative to reversed) gradient, bumblebees make shorter inter-floral flights while moving upwards (potentially visiting more female-phase flowers). Furthermore, after foraging on a reversed nectar gradient, bumblebees entered the next inflorescence closer to the middle of the display—potentially missing female-phase flowers altogether.

My size-dependent hypothesis for the function of the nectar gradient, however, focuses precisely on the efficiency of pollen removal from the display. Structured dichogamy itself may improve pollen export by limiting the amount of pollen that is lost to stigmas within the same plant, as pollinators ascend the display (Harder et al., 2000, Routley and Husband, 2003). This mechanism cannot, however, mediate the amount of exposed pollen removed by individual pollinators. The largest, most attractive displays in a population should benefit from any mechanism that maintains or increases attractiveness while also limiting the number of pollen-bearing flowers probed per visit. Smaller displays minimize their disadvantage by dispensing pollen in larger amounts.

Observed variation in the nectar distribution of *C. angustifolium* inflorescences, and its predicted effect on per-visit pollen removal, supports the size-dependent hypothesis. Small inflorescences distributed nectar nearly evenly among female- and male-phase flowers, whereas larger inflorescences allocated extra nectar to female-phase flowers. In my (*gradient strength*) experiment, bumblebees probed fewer male-phase flowers per visit when nectar was distributed in a strong, relative to weak, gradient. This behaviour is consistent with known departure rules, which appear to be based on a threshold nectar volume at the current (e.g. Hodges 1985) or previous two (Kadmon and Shmida, 1992) flowers probed. On large *C. angustifolium* inflorescences in nature, I expect that bumblebees should (on average) probe fewer male-phase flowers per visit than they would if nectar production was allocated equally among flowers. Small inflorescences, on the other hand, are visited less frequently than larger conspecifics, yet their male-phase flowers produce nectar at a similar rate. Hence, if more nectar can accumulate between visits to small inflorescences, then bees are likely to visit several male-phase flowers per visit and remove relatively large pollen loads from each flower probed.

The proportion of open flowers probed per visit is in fact negatively related to floral display size in *C. angustifolium* (Schmid-Hempel and Speiser, 1988, JMB unpub. data), as predicted by models of pollen export maximization (Iwasa et al. 1995). Unfortunately, it is difficult to weigh the relative effects of slight nectar differences and other factors that may contribute to this behaviour (see Ohashi and Yahara, 1999). Nevertheless, I have extended the hypothesis (Biernaskie and Cartar 2004) that plants can mediate the amount of pollen removed per visit by manipulating the patch departure behaviour of pollinators.

Nectar production as a component of size-dependent gender allocation

On average, the smallest ramets in *C. angustifolium* populations produced nearly three times more flowers per gram of ramet biomass than the largest ones, but matured fewer seeds per flower. It seems likely that the low seed set in small ramets is a consequence of seed abortion rather than pollen limitation. At my sites, pollinators typically visit inflorescences at mean rates greater than once per minute (JMB, unpub. data). Furthermore, in other populations of *C. angustifolium*, female-phase flowers of both large and small floral displays receive more than ample pollen loads for full seed set in only hours of open pollination (Schmid Hempel and Speiser 1988). Thus, by the simplified measure of flower (male) and seed (female) production alone, small ramets seem to emphasize male function whereas larger ramets emphasize female function.

My estimate of gender modification is consistent with available estimates from other animal-pollinated angiosperms. The observed relation between ramet biomass and total seed production in *C. angustifolium* was admittedly imprecise, but statistically indistinguishable from all equivalent estimates reviewed by Klinkhamer and deJong (1997). The relation between ramet biomass and total flower production ($b^* = 0.64$ flowers/g) was also similar to available data (in that most estimates are < 1); my estimate, however, is among the lowest reported (mean = 0.88, std. dev. = 0.19; n = 8 species; Klinkhamer and de Jong 1997). This comparison implies that large *C. angustifolium* ramets strongly limited their allocation to male reproduction—a surprising result if large, attractive plants can use pollen-dispensing mechanisms to minimize the costs of excess pollen removal (above).

Recognize, however, that simplified measures of gender allocation (flower and gamete production) ignore additional investments that can modify size-specific gender. In particular, if seed set is not pollen limited—as assumed here (and see Weins et al., 1987)—then pollinator attraction characters may be assigned mainly to the male function (e.g. Charnov and Bull, 1986). In *C. angustifolium*, the total allocation to nectar production increased with plant (and display) size, partly owing to the increased NPR of female-phase flowers (Fig.1). And because the rate of bumblebee visitation is often positively related to a plant's rate of nectar production (e.g. Dreisig, 1995, Thomson, 1988), allocation to nectar may increase the attractiveness of large displays. I propose that an increased NPR in female-phase flowers may be the most efficient way to increase the rate of pollinator visits the inflorescence (and male fitness) without exposing additional pollen to excess removal (essentially increasing the 'quality' of existing pollen grains; Charnov and Bull 1986). Hence, a measure of investment based on flower production alone could mask the true allocation to male reproduction—even an emphasis on male reproduction—in the largest ramets of *C. angustifolium* populations.

Models that assume a linear female fitness gain curve and diminishing returns to male investment predict a constant absolute investment into male function for individuals above a threshold size (e.g. Yamaguchi, 1985, Zhang and Jiang, 2002). Given that my estimates of male investment (flower and nectar production) cannot be assigned exclusively to the male function, this remains a difficult prediction to test for *C. angustifolium*. One seemingly supportive example (Zhang and Jiang 2002) comes from monecious populations of *Sagittaria latifolia*; in that study, female, but not male, flower production increased steadily with ramet size (Sarkissian, et al., 2001). One must be careful, however, to assume that female flower production serves only a female function. The addition of female flowers to a monecious floral display may be another efficient mechanism to increase inflorescence attractiveness without paying the costs of excessive pollen removal. As above, this viewpoint highlights the fundamental role of the entire floral display as a single functional mating unit (Harder et al. 2001, and see Elle and Meagher, 2000).

Conclusion. I identified reproductive allocations of an animal-pollinated plant that vary with correlates of resource state and, presumably, with reproductive winnings. I successfully predicted size-dependent variation in the pattern of nectar production in *C. angustifolium*, and suggest that its effects on bumblebee visitation and pollen removal are designed to maximize pollen export from the display. This evidence offers a particularly unique example of how flowering plants might adaptively 'manipulate' the foraging behaviour of their animal pollinators.

LITERATURE CITED

Best, L.S. and Bierzychudek, P. 1982. Pollinator foraging on foxglove (*Digitalis purpurea*): A test of a new model. *Evolution*, **36**: 70-79.

Biernaskie, J.M. and Cartar, R.V. 2004. Variation in rate of nectar production depends on floral display size: a pollinator manipulation hypothesis. *Functional Ecology*, **18**: 125-129.

Chaplin, S.J. and Walker, J.L. 1982. Energetic constraints and adaptive significance of the floral display of a forest milkweed. *Ecology*, **63**: 1857-1870.

Charnov, E.L. 1982. *The Theory of Sex Allocation.* Princeton, NJ: Princeton University Press.

Charnov, E.L. and Bull, J.J. 1986. Sex allocation, pollinator attraction and fruit dispersal in cosexual plants. *Journal of Theoretical Biology*, **118**: 321-325.

Corbet, S.A., Cuthill, I., Fallows, M., Harrison, T. and Hartley, G. 1981. Why do nectar-foraging bees and wasps work upwards on inflorescences? *Oecologia*, **51**: 79-83.

Dreisig, H. 1995. Ideal free distributions of nectar foraging bumblebees. *Oikos*, **72**: 161-172.

Dudash, M.R. 1991. Plant size effects on female and male function in hermaphrodite *Sabatia angularis* (Gentianaceae). *Ecology*, **72**: 1004-1012.

Elle, E. and Meagher, T.R. 2000. Sex allocation and reproductive success in the andromonoecious perennial *Solanum carolinense* (Solanaceae). II. Paternity and functional gender. *American Naturalist*, **156**: 622-636.

Harder, L.D. and Thomson, J.D. 1989. Evolutionary options for maximizing pollen dispersal in animal-pollinated plants. *American Naturalist*, **133**: 323-344.

Harder, L.D. 1990. Pollen removal by bumble bees and its implications for pollen dispersal. *Ecology*, **71**: 1110-1125.

Harder, L.D., Barrett, S.C.H. and Cole, W.W. 2000. The mating consequences of sexual segregation within inflorescences of flowering plants. *Proc. R. Soc. Lond. B*, **267**: 1-6.

Harder, L.D., Williams, N.M., Jordan, C.Y. and Nelson, W.A. 2001. The effects of floral design and display on pollinator economics and pollen dispersal. In *Cognitive Ecology of Pollination* (Thomson, J.D., eds), pp. 297-317. Cambridge University Press.

Hodges, C.M. 1985. Bumblebee foraging: the threshold departure rule. *Ecology*, **66**: 179-187.

Ishii, H.S. 2004. Increase of male reproductive components with size in an animal-pollinated hermaphrodite, *Narthecium asiaticum* (Liliaceae). *Functional Ecology*, **18**: 130-137.

Iwasa, Y., de Jong, T.J. and Klinkhamer, P.G.L. 1995. Why pollinators visit only a fraction of the open flowers on a plant: The plant's point of view. *Journal of Evolutionary Biology*, **8**: 439-453.

Kadmon, R. 1992. Dynamics of forager arrivals and nectar renewal in flowers of *Anchusa strigosa*. *Oecologia*, **92**: 552-555.

Kadmon, R. and Shmida, A. 1992. Departure rules used by bees foraging for nectar: a field test. *Evolutionary Ecology*, **6**: 142-151.

Klinkhamer, P.G.L., deJong, T.J. and Metz, A.J. 1994. Why plants can be too attractive--A discussion of measures to estimate male fitness. *Journal of Ecology*, **82**: 191-194.

Klinkhamer, P.G.L. and deJong, T.J. 1997. Size-dependent allocation to male and female reproduction. In *Plant Resource Allocation* (Grace, J., eds), pp. 211-230. San Diego: Academic Press.

Klinkhamer, P.G.L., deJong, T.J. and Metz, A.J. 1997. Sex and size in cosexual plants. *Trends in Ecology and Evolution*, **12**: 260-265.

Levy, P.S. and Lemeshow, S. 1999. *Sampling of Populations.* Toronto: John Wiley and Sons, Inc.

Lloyd, D.G. and Bawa, K.S. 1984. Modification of the gender of seed plants in varying conditions. *Evolutionary Biology*, **17**: 255-338.

Ohashi, K. and Yahara, T. 1999. How long to stay on, and how often to visit a flowering plant? A model for foraging strategy when floral displays vary in size. *Oikos*, **86**: 386-392.

Ohashi, K. and Yahara, T. 2001. Behavioural responses of pollinators to variation in floral display size and their influences on the evolution of floral traits. In *Cognitive Ecology of Pollination* (Thomson, J.D., eds), pp. 274-296. Cambridge: Cambridge University Press.

Orth, A.I. and Waddington, K.D. 1997. The movement patterns of carpenter bees *Xylocopa micans* and bumblebees *Bombus pennsylvanicus* on *Pontederia cordata* inflorescences. *Journal of Insect Behavior*, **10**: 79-86.

Pyke, G.H. 1978. Optimal foraging in bumblebees and coevolution with their plants. *Oecologia*, **36**: 281-293.

Routley, M.B. and Husband, B.C. 2003. The effect of protandry on siring success in *Chamerion angustifolium* (Onagraceae) with different inflorescence sizes. *Evolution*, **57**: 240-248.

Sargent, R.D. and Roitberg, B.D. 2000. Seasonal decline in male-phase duration in a protandrous plant: a response to increased mating opportunities? *Functional Ecology*, **14**: 484-489.

Sarkissian, T.S., Barrett, S.C.H. and Harder, L.D. 2001. Gender variation in *Sagittaria latifolia* (Alismataceae): is size all that matters. *Ecology*, **82**: 360-373.

Schmid-Hempel, P. and Speiser, B. 1988. Effects of inflorescence size on pollination in *Epilobium angustifolium. Oikos*, **53**: 98-104.

Solbrig, O.T. and Solbrig, D.J. 1984. Size inequalities and fitness in plant populations. In *Oxford Surveys in Evolutionary Biology* (Ridley, M., eds), pp. 141-159. Oxford University Press.

Thomson, J.D. 1988. Effects of variation in inflorescence size and floral rewards on the visitation rates of traplining pollinators of *Aralia hispida. Evolutionary Ecology*, **2**: 65-76.

Waddington, K.D. and Heinrich, B. 1979. The foraging movements of bumblebees on vertical "inflorescences": An experimental analysis. *Journal of Comparative Physiology*, **134**: 113-117.

Weins, D., Calvin, C.L., Wilson, C.A., Davern, C.I., Frank, D. and Seavey, S.R. 1987. Reproductive success, spontaneous embryo abortion, and genetic load in flowering plants. *Oecologia*, **71**: 501-509.

Williams, G.C. 1992. *Natural Selection: Domains, Levels, and Challenges.* New York: Oxford University Press.

Worley, A.C., Baker, A.M., Thompson, J.D. and Barrett, S.C.H. 2000. Floral display in *Narcissus*: Variation in flower size and number at the species, population, and individual levels. *International Journal of Plant Sciences*, **161**: 69-79.

Zhang, D.-Y. and Jiang, X.-H. 2002. Size-dependent resource allocation and sex allocation in herbaceous perennial plants. *Journal of Evolutionary Biology*, **15**: 74-83.

FIGURES

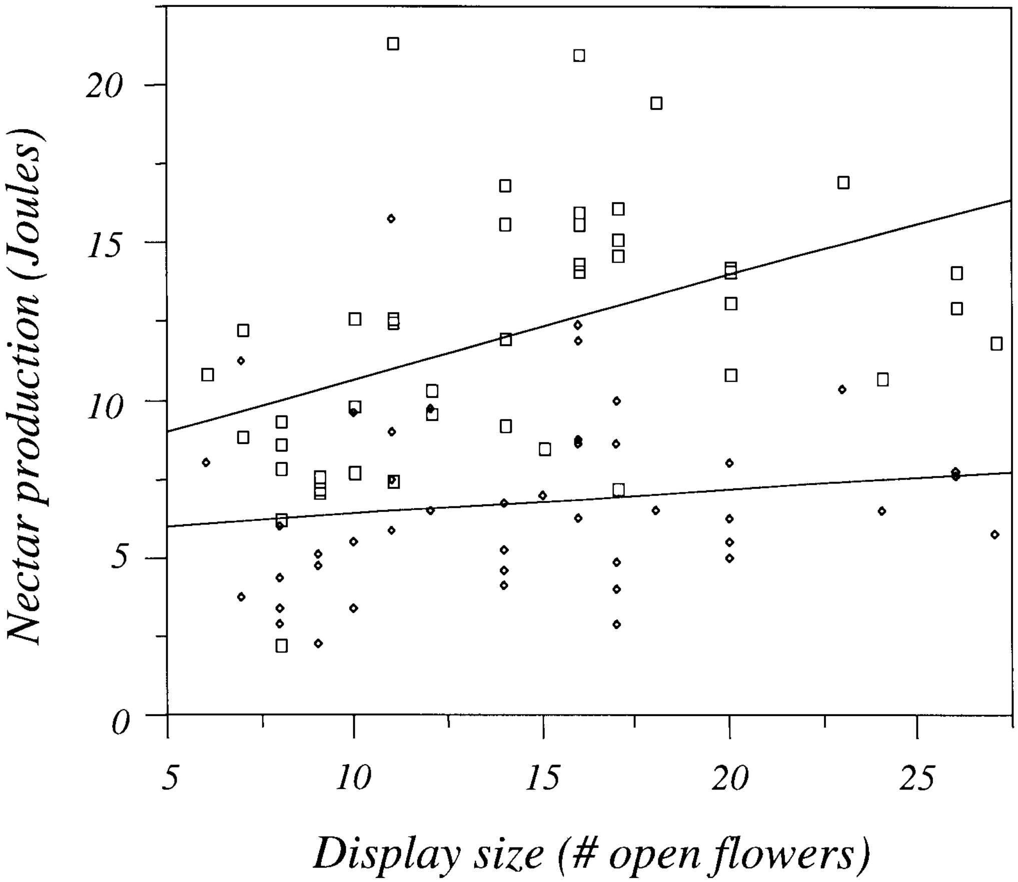


Figure 3-1 Interaction of floral display size and gender-phase (◊ = male phase, □ = female phase) on the mean 4 hour nectar production of C. angustifolium flowers. Although raw data are plotted, analyses in the text accounted for having two measures (male- and female-phase production) within each ramet.

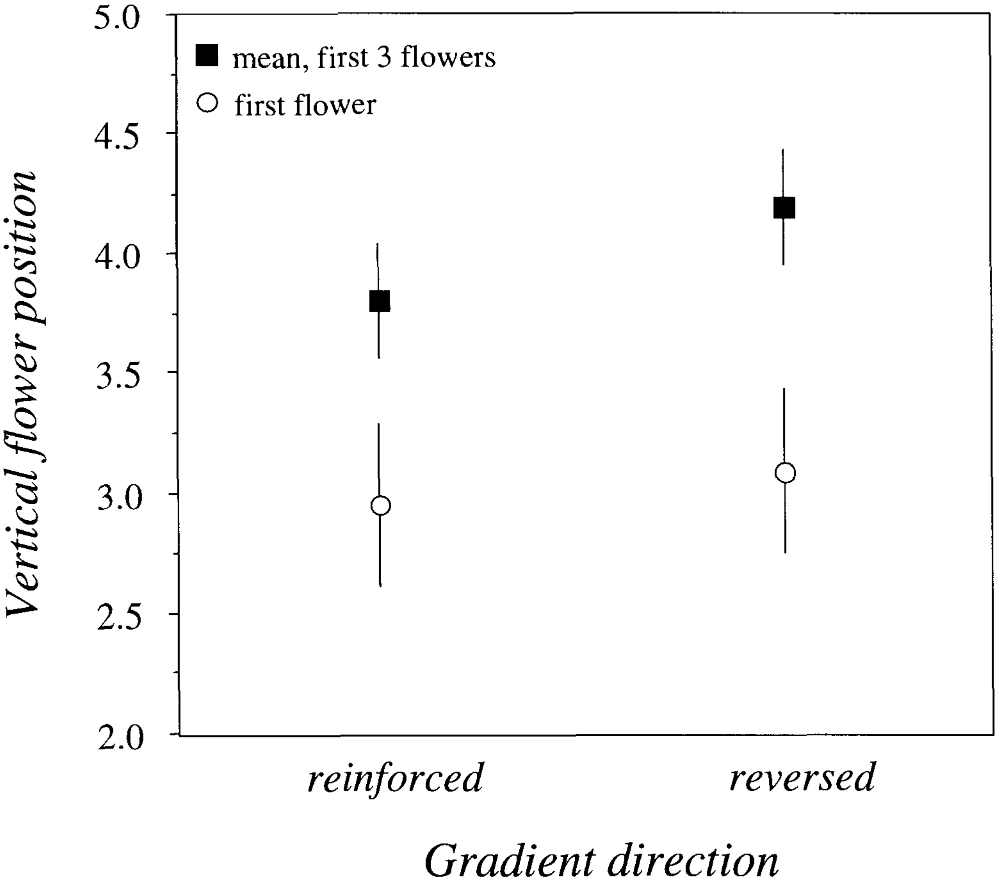


Figure 3-2 Mean (± 95% C.I.) flower positions probed by bumblebees on 10-flowered displays with either a 'reinforced' or 'reversed' nectar gradient added (see text). Estimated differences: position of first flower probed: 0.25 flowers (-0.36, 0.86); mean position of the first three flowers probed: 0.55 flowers (0.17, 0.93).

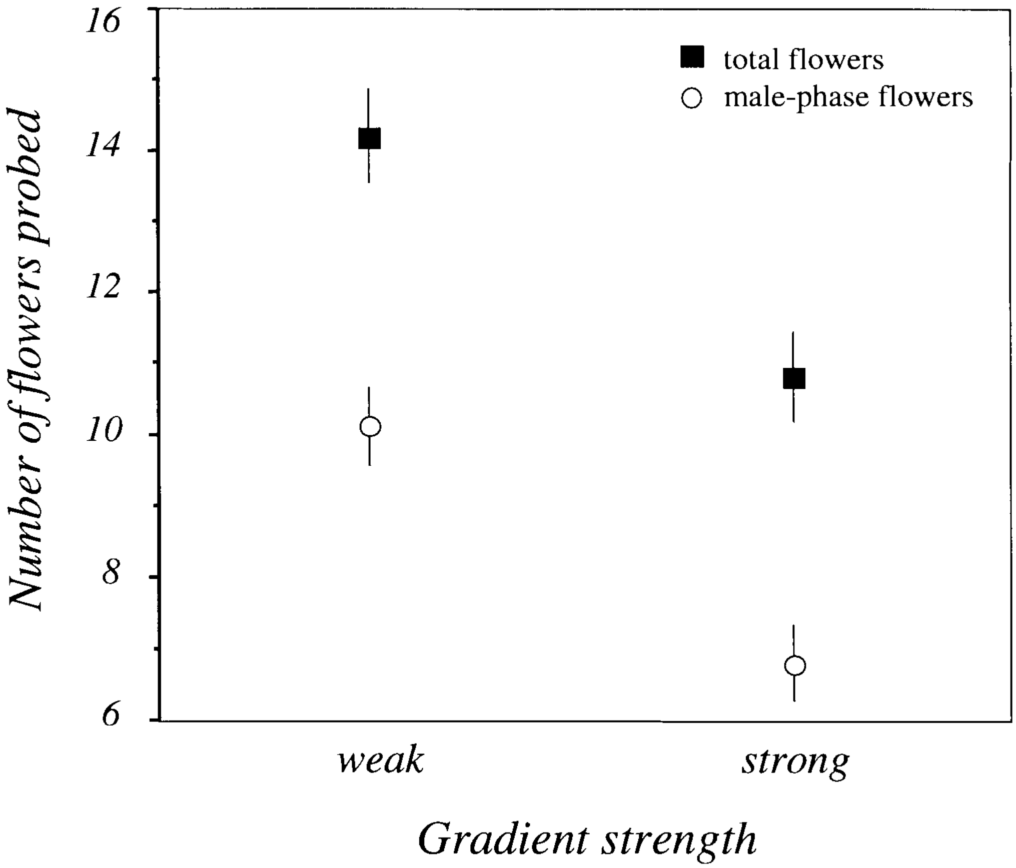
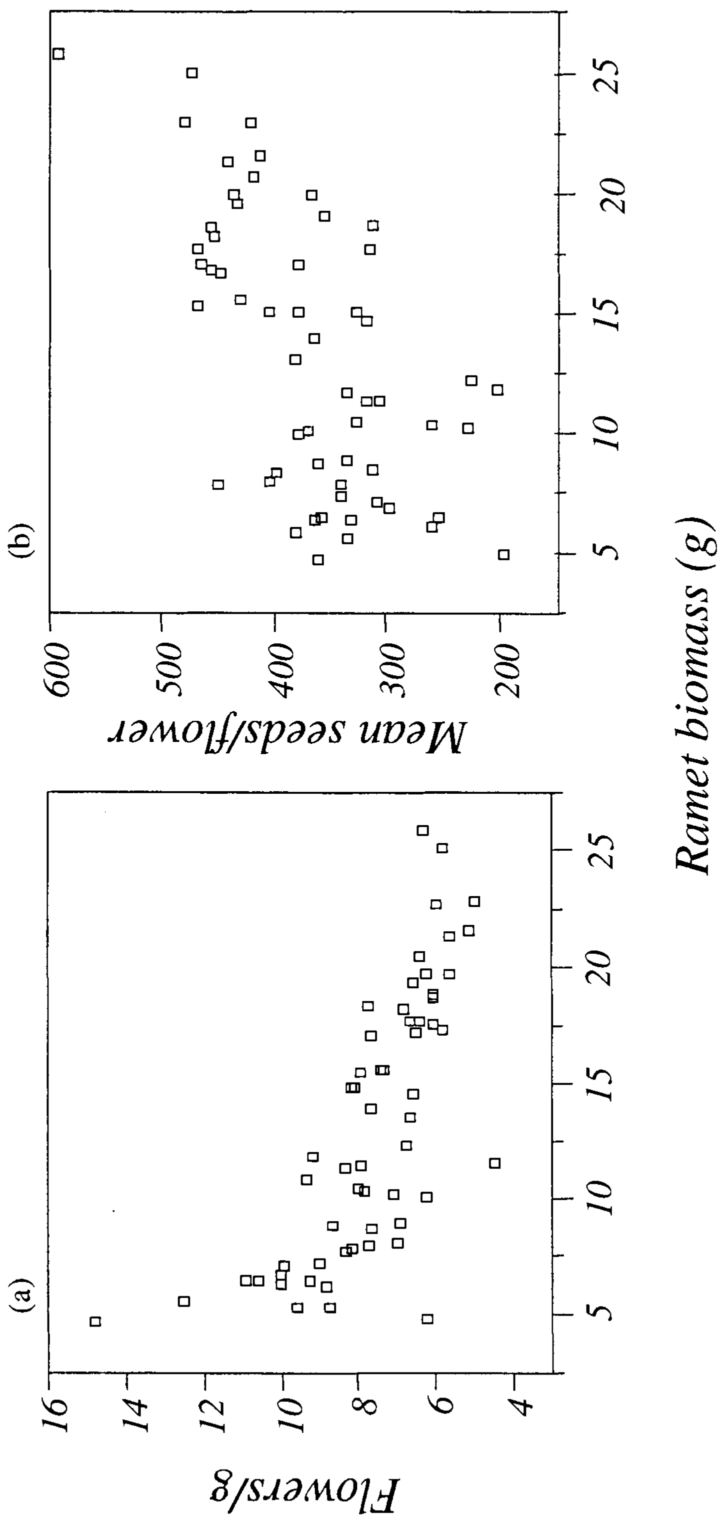


Figure 3-3 Least squares mean (± 95% C.I.) number of flowers probed by bumblebees on 11-flowered displays with alternative nectar gradients added (see text). Estimated differences: total flowers probed: 3.38 flowers (2.41, 4.35); number of male-phase flowers probed: 3.24 flowers (2.43, 4.04).

Figure 3-4 Leverage plots of the relation between ramet biomass and (a) flower production/g biomass and (b) mean seed number/flower in *C. angustifolium*. The mean regression slope across all transects was -0.23 (-0.35, -0.11) for (a) and 9.04 (3.69, 14.39) for (b).


(a)
(b)
Flowers/g
16
14
12
10
8
6
4
Mean seeds/flower
600
500
400
300
200
5
10
15
20
25
Ramet biomass (g)

TABLES

Table 3-1. Estimated slopes (*b*) in the regression: log y = a + b log x , where x = ramet biomass (grams) and y = total flower, fruit, or seed production of *C. angustifolium* ramets. The slope was calculated for each randomly selected transect (from n = 10 ramets) and the mean is taken as the overall effect size (b^*).

Response Variable (y)	Transect 1	 2	 3	 4	 5	 6	b^* (95% C.I.)
total # flowers	0.66	0.58	0.75	0.71	0.72	0.44	**0.64** **(0.52, 0.77)**
total # fruits	0.65	0.57	0.76	0.74	0.74	0.5	**0.66** **(0.55, 0.77)**
total # seeds	1.05	0.73	1.11	1.24	0.83	0.58	**0.92** **(0.65, 1.19)**

CHAPTER 4 GENERAL CONCLUSIONS

The adaptive significance of floral phenotypes in animal-pollinated plants must often be related to their influence on pollinator foraging behaviour. Thus, it is necessary to understand how variant phenotypes influence: (1) attraction of pollinators to the plant, (2) the number and sequence of flowers visited within a plant, and (3) the behaviour of pollinators upon leaving the plant (Waser, 1983, Zimmerman, 1988). While employing this basic framework, I sought a more precise understanding of the types of selection that have shaped nectar production phenotypes in *Chamerion anugustifolium.* I considered how the optimal investment to nectar production may depend on the investment strategies of other plants in the population (Chapter 2), and how the optimal distribution of nectar within inflorescences may depend on the plant's size, or resource state (Chapter 3).

Social competition in flowering plants: a pollinator attraction game

I outlined a game-theoretic approach to ask: how much energy should a plant invest into reward production? The ESS investment to rewards may be considerable if the disadvantage of competing against more rewarding conspecifics is great. This competitive disadvantage may be realized through a reduced rate of pollinator visits to the plant or through a reduced number of flowers probed per visit.

My original goal was to determine how pollinator visitation rates to a given plant depend the relative rate of nectar production. Using defoliation treatments, I altered the nectar production rate (NPR) of small patches of inflorescences, while monitoring bumblebee visitation to competing (control) patches before and after manipulation, over a series of days. These experiments were unsuccessful, however, due to an unforeseen confound of treatment with time; I was unable to determine whether shifts in pollinator visitation rate to the control patches were due to competitive interactions, or to temporal changes in pollinator abundance. Instead, I pursued a much simpler experiment, in which I added nectar to inflorescences and observed competitive interactions over a relatively short time period. These experiments, and their limitations, were discussed in Chapter 2.

Future experiments must be designed with proper controls to account for changes in bumblebee abundance over time. It would be particularly interesting to determine how pollinator visitation rates to high and low nectar producers depends on the presence of the highly rewarding phenotype at varying frequency or density. Finally, a potentially useful alternative to experimental manipulation may be to observe how the visit rate to a given inflorescence depends on its NPR *relative* to the rate of production measured in the local environment.

Although my empirical studies were limited in scope, the model of social competition for pollinator service remains a useful concept. The competitive interactions assumed in this model are potentially important factors that influence the mean reward production of a population and the maximum reward production within populations. Furthermore, I suggested that success in social competition for access to pollinators is analogous to the sexual selection pressures that favour extravagance in animal mating characters. It is an exciting prospect to describe floral display characters that are truly comparable to the costly signals and weaponry known in animals.

Gender and rewards vary with competitive ability in an animal-pollinated plant

Recent models of pollen export maximization predict that the proportion of pollen-bearing flowers probed per visit should be negatively related to floral display size (e.g. Iwasa, et al., 1995). Throughout Chapter 3, I made use of a simplified optimality criterion: that the largest floral displays in the population should limit the number of male-phase flowers probed per visit more so than smaller (less attractive) conspecifics. This allowed me to predict the observed size-dependent variation in the vertical nectar distribution of *C. angustifolium*, and to infer the fitness consequences of variant nectar gradients with respect to floral display size.

The vertical nectar gradient in inflorescences with structured dichogamy is one of the most readily cited examples of how plant phenotypes can manipulate pollinator behaviour to effect pollen transfer (Rathcke, 1992, Zimmerman, 1988). I used the size-dependent optimality criterion to more precisely understand how and why the nectar gradient influences the number of flowers probed per visit. My data suggest that the nectar distribution can be adjusted facultatively to mediate the pollen removal of individual visitors, depending on attractiveness to pollinators. I concluded that an important function of the nectar gradient is to limit the number of pollen-bearing flowers probed per visit. Overall, Chapter 3 presents one of the most comprehensive examples of how floral phenotypes might manipulate the foraging behaviour of pollinators to the plant's advantage.

LITERATURE CITED

Iwasa, Y., de Jong, T.J. and Klinkhamer, P.G.L. 1995. Why pollinators visit only a fraction of the open flowers on a plant: The plant's point of view. *Journal of Evolutionary Biology*, **8**: 439-453.

Rathcke, B.J. 1992. Nectar distributions, pollinator behavior, and plant reproductive success. In *Effects of Resource Distribution on Animal-plant Interactions* (Hunter, M.D., Ohgushi, T. and Price, P.W., eds), pp. 114-138. Academic Press.

Waser, N.M. 1983. The adaptive nature of floral traits: ideas and evidence. In *Pollination Biology* (Real, L.A., eds), pp. Orlando: Academic Press.

Zimmerman, M. 1988. Nectar production, flowering phenology, and strategies for pollination. In *Plant Reproductive Ecology: Patterns and Strategies* (Lovett Doust, J.L. and Lovett Doust, L., eds), pp. 157-178. New York: Oxford University Press.

APPENDIX

HANDLING TIME OF MALE- AND FEMALE-PHASE *CHAMERION ANGUSTIFOLIUM* FLOWERS

I.1. Difference between gender phases. The length of time that a nectar-foraging pollinator stays on a flower (handling time) should depend on the amount of nectar available in that flower and on any floral character that affects the rate at which nectar can be extracted. Handling time is positively related to the amount of pollen removed (Harder, 1990, Young and Stanton, 1990) and deposited (Galen and Plowright, 1985) during a single flower visit. In most animal-pollinated species, flowers expose only a small amount of pollen at any one time. Flowers of *Chamerion angustifolium*, for example, limit the amount of exposed pollen through sequential anther dehiscence (Sargent, 2003). If this is interpreted as an adaptation to limit pollen removal by each individual pollinator, then one might expect flowers in the male phase to further restrict pollen removal by *decreasing* their handling time. On the other hand, plant fitness may benefit if female-phase flowers can maximize the amount of pollen transferred from pollinator bodies to the stigmatic surface during each flower visit—not only to ensure pollen deposition (female fitness), but also to remove it from the pool of competing pollen grains (male function) (Charnov, 1982, p. 259). Thus, female-phase flowers may have special mechanisms to *increase* their handling time.

I predicted that if an equivalent volume of nectar was added to male- and female-phase flowers, then it should take bumblebees longer to extract the nectar reward from female-phase flowers.

Methods. At Silver Star Mountain, *C. angustifolium* inflorescences were haphazardly chosen for nectar enrichment. At a given inflorescence, 42% sucrose solution ('nectar') was added to either male- or female- phase flowers, in one of three volumes: 0.5 μl, 1.0 μl, or 2.0 μl (6 inflorescences for each gender phase-volume combination). Once an inflorescence had been assigned to a gender-phase and nectar volume, I selected up to three male- or female-phase flowers (as defined in chapter 3) for observation. Flowers were emptied of any standing nectar with a 2 μl microcapillary tube before adding the appropriate volume of nectar. To access the nectary of male-phase flowers, I removed one stamen from the underside of male-phase flowers; this did not appear to affect bees' foraging behaviour. During a bumblebee visit to the inflorescence, I recorded the handling time (time between landing and leaving a flower; measured with a stopwatch) of each enriched flower probed, and later calculated a mean handling time per visit. I checked whether enriched flowers had been completely emptied, and discarded data from those that were not (a rare event). Focal flowers were refilled and observations continued for six consecutive bumblebee visits. I calculated a mean floral handling time for each inflorescence to estimate the mean handling time of each gender phase-nectar volume combination.

Contrary to expectation, the handling time of male-phase flowers was longer than the handling time of female-phase flowers at each level of nectar-enrichment (Fig. 1), and hence, these observations disproved my functional hypothesis. There are at least two proximate explanations for this unexpected result: (1) bumblebees may collect both nectar and pollen at male-phase flowers, which takes extra time, or (2) some intrinsic feature of male-phase flowers (relative to female-phase flowers) makes nectar more difficult to extract (or vice versa).

I.2. A mechanistic explanation. I hypothesized that it is more difficult for bumblebees to access the nectary of male-phase flowers than it is on older female-phase flowers. Young pollen-bearing flowers have filaments that are tightly arranged around the nectary, whereas the filaments of late-stage (female-phase) flowers are wilted and easily circumvented.

I predicted that if male-phase flowers were manipulated to facilitate access to the nectary, then manipulated male-phase flowers and (unmanipulated) female-phase flowers would have equivalent handling times.

Methods. On each of eight inflorescences, I selected one female-phase and two male-phase flowers for observation. One of the male-phase flowers was randomly assigned to an 'open' treatment, in which I completely removed the top four filaments (where bumblebees typically probe for nectar). This treatment greatly increased bees' access to the nectary, and in this respect, made male- and female-phase flowers similar in appearance. To all three focal flowers, I added 2 µl of nectar. All pollen was removed from male-phase flowers (both 'open' and control) by clipping off dehisced anthers. I recorded the handling time of each focal flower for five inflorescence visits in which a bee probed all three focal flowers. As above, nectar was refilled between visits.

Because individual bees differ consistently in time spent at a given flower (see below), I considered each bee as the unit of replication in this experiment. Hence, I estimated the mean handling time of each floral type while using 'bee' as a (random) blocking variable. Differences between means were tested with Tukey-Kramer independent contrasts.

Results. As expected, there were consistent differences among the mean handling time of individual bees ($F_{1,78} = 5.15$, $P < 0.0001$). After accounting for this variation, the estimated handling time of the 'open' flower type was significantly less than unmanipulated male-phase flowers (Fig. 2; estimated difference: 2.85 seconds (1.95, 3.76); $F_{1,78} = 56.6$, $P < 0.0001$). However, the mean handling time of 'open' flowers was still slightly greater than that of female-phase flowers (est. difference: 0.95 seconds (0.04, 1.86); $F_{1,78} = 18.04$, $P = 0.014$).

These results suggest, first, that the extra time spent on male-phase *C. angustifolium* flowers was likely not a consequence of pollen collection by visiting bumblebees. There was no pollen available in the current experiment, yet bees spent nearly twice as long at male-phase flowers than at female-phase flowers with an equivalent nectar volume (comparable to data in Fig. 1). Second, my experiment suggests that access to the nectary is not the only proximate explanation for the handling time difference between the gender phases of *C. angustifolium* flowers. One potential mitigating factor might be the large stigma of female-phase flowers that could act as a 'landing pad' for bumblebees. Nevertheless, it is currently unclear whether the extra handling time of male-phase flowers is the effect of a developmental constraint or of an unknown adaptation.

LITERATURE CITED

Charnov, E.L. 1982. *The Theory of Sex Allocation.* Princeton, NJ: Princeton University Press.

Galen, C. and Plowright, R.C. 1985. The effects of nectar level and flower development on pollen carry-over in inflorescences of fireweed (*Epilobium angustifolium*) (Onagraceae). *Canadian Journal of Botany*, **63**: 488-491.

Harder, L.D. 1990. Pollen removal by bumble bees and its implications for pollen dispersal. *Ecology*, **71**: 1110-1125.

Sargent, R.D. 2003. Seasonal changes in pollen-packaging schedules in the protandrous plant *Chamerion angustifolium. Oecologia*, **135**: 221-226.

Young, H.J. and Stanton, M.L. 1990. Influences of floral variation on pollen removal and seed production in wild radish. *Ecology*, **71**: 536-547.

FIGURES

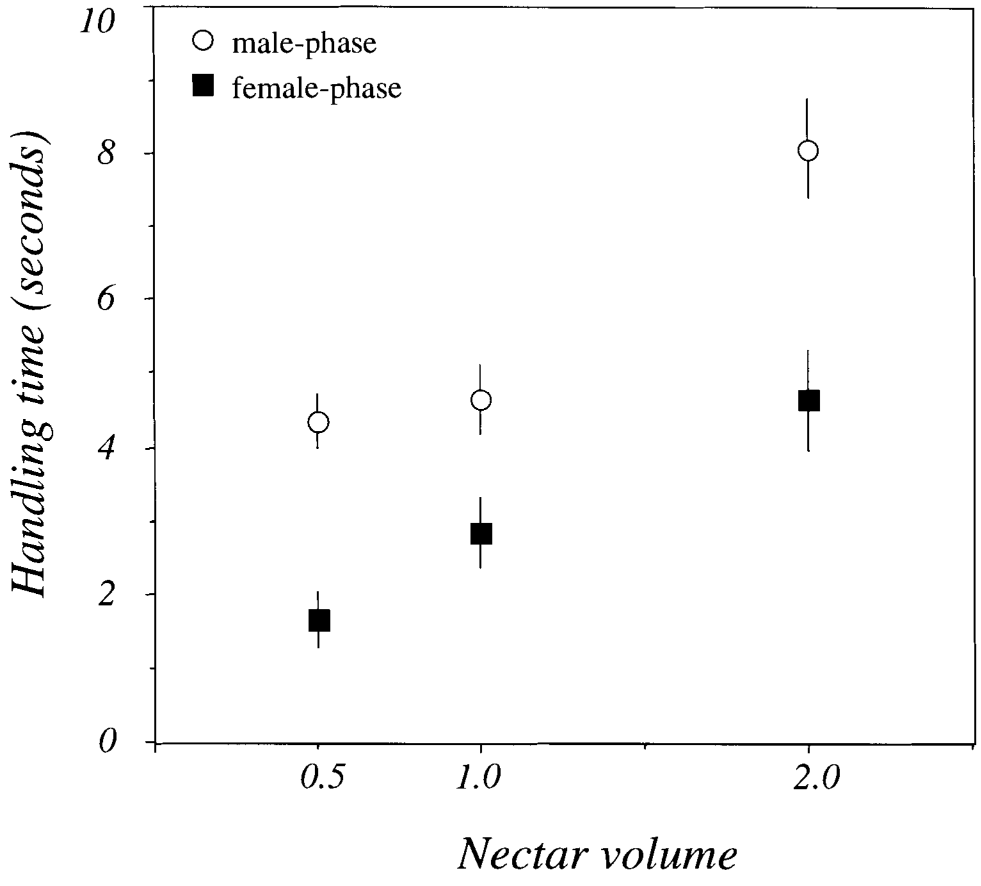


Figure A-1 Mean (± 95% C.I.) time spent by nectar-collecting bumblebees at male- and female-phase Chamerion angustifolium flowers with three controlled volumes of nectar. Handling time of male-phase flowers was significantly longer at all three nectar levels. The estimated differences between gender phases were not detectably different among the three levels of nectar volume (0.5 ml: 2.7 seconds (2.1, 3.3); 1.0 ml: 1.8 sec. (1.1, 2.6); 2.0 ml: 2.3 sec. (1.4, 3.3)).

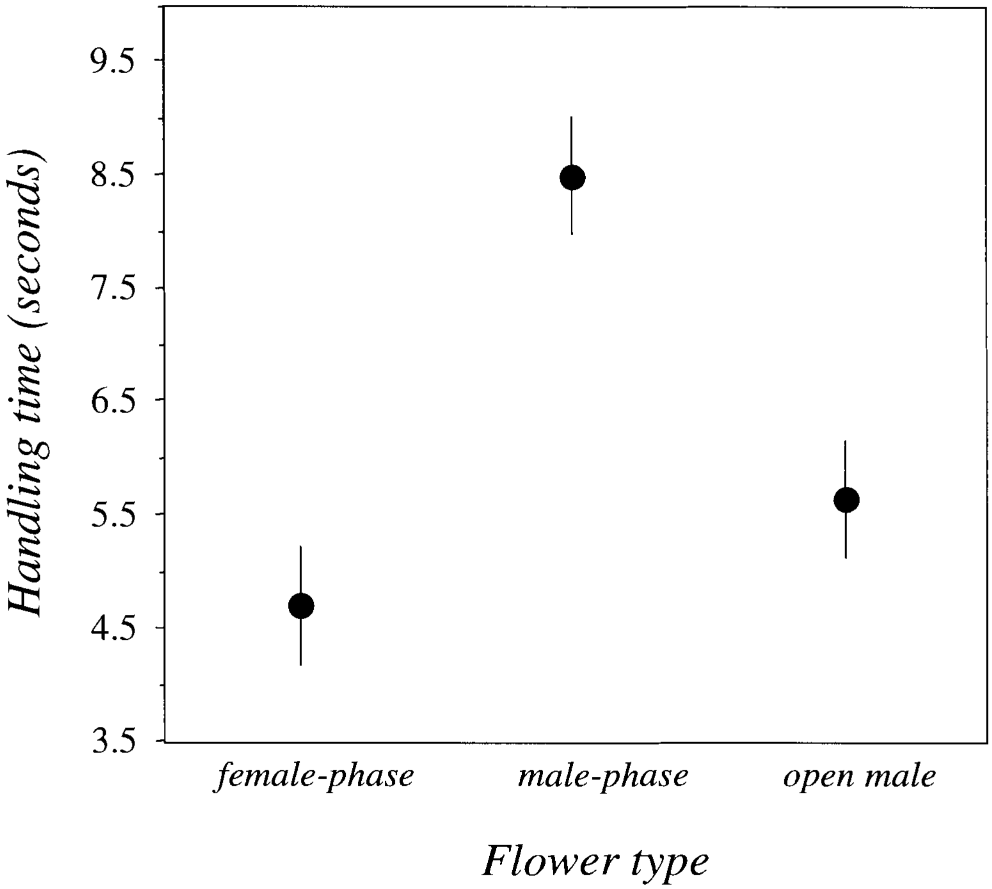


Figure A-2 Least squares mean (± 95% C.I.) time spent by nectar-collecting bumblebees at three flower types that contained 2 ml of nectar. The 'open male' type was meant to resemble the female-phase flower, with respect to access to the nectary. The estimated mean handling time of 'open male' flowers was, however, significantly different from both the (unmanipulated) male- and female-phase flower types.