Floral traits, pollinator behavior, and plant reproduction: tests of natural and sexual selection in the hummingbird-pollinated herb Chrysothemis friedrichsthaliana

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FLORAL TRAITS, POLLINATOR BEHAVIOR, AND PLANT REPRODUCTION: TESTS OF NATURAL AND SEXUAL SELECTION IN THE HUMMINGBIRD-POLLINATED HERB *CHRYSOTHEMIS FRIEDRICHSTHALIANA*

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree in Doctor of Philosophy in The Department of Biological Sciences

by Jane Elizabeth Carlson  
B.S., Cornell University, 2002
August 2007
Acknowledgements

This dissertation was only possible because I was aided by many excellent scientists, students and collaborators. Foremost among these is my major professor, Kyle Harms. Kyle allowed me to think and work independently, yet was unfailing in his interest, encouragement and support. If future co-workers have only a few of the professional mannerisms that so distinctly characterize Kyle, I shall consider myself extremely fortunate to be a scientist.

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Abstract

Pollinators may promote floral evolution by selectively visiting preferred floral phenotypes. Herbivores may also influence the evolution of floral traits, by reducing the fitness of poorly-defended plants, such as those without protective sepals. In this dissertation, I investigate the reproductive and evolutionary consequences of floral visitors for two floral traits of the Neotropical herb *Chrysothemis friedrichsthaliana* (Gesneriaceae). I focus on the trait male-biased nectar production, which results from coincident temporal shifts in nectar production rates and sexual function within flowers. First, I review theory relevant to male- and female-biased nectar production, and I generate hypotheses to explain its evolution in 41+ representative species. Next, I test natural and sexual selection hypotheses using *C. friedrichsthaliana* and its hummingbird pollinator *Phaethornis striigularis* (Trochilidae). Sexual selection theory predicts that secondary sexual traits (like nectar) serve to increase male more than female pollination success, and in accordance, (1) nectar production was consistently greater during the male phase; (2) visits by *P. striigularis* were sometimes limiting, indicating that plants had to compete for visits; (3) hummingbirds made more and longer visits to unmanipulated male- versus female-phase flowers; (4) female fecundity was maximized by one visit, whereas male fecundity continued to increase with additional visits. Using nectar manipulations, I also showed that *P. striigularis* discriminated against unrewarding real and artificial flowers, suggesting that preferences are based partially on nectar. Natural selection for inbreeding avoidance predicts costs of inbreeding and specific pollinator responses to reward distributions, yet neither prediction was well supported in *C. friedrichsthaliana*. Thus, sexual selection, more so than inbreeding avoidance, likely maintains male-biased nectar production in this species. I used a second floral trait, the cup-like, liquid-filled calyx of *C. friedrichsthaliana*, to test the hypothesis
that sepal morphology functions predominately for herbivore defense. Experimental calyx draining showed that liquid barriers over buds halved flower loss to a highly detrimental microlepidopteran herbivore (Alucitidae). Thus, C. friedrichthaliana’s water calyx promotes reproduction by protecting developing buds. This dissertation is the first to comprehensively describe the mechanistic basis for both traits’ functions. It also lays the groundwork for future, novel explorations into natural and sexual selection on flowers.
Chapter 1
Introduction

Flowers have attracted the attention of scientists, naturalists, and artists for centuries. Detailed studies of flowers were first recorded in 500 BC, in the form of decorative botanical illustrations (Blunt, 1994). Since the late 16th century, naturalists and botanists have relied heavily on flowers in plant species descriptions and taxonomy, paying tribute to both the attractive and biological functions of flowers (e.g., Lloyd and Barrett, 1996). That visually-oriented animals, such as humans, find flowers attractive is not accidental; most flowers have evolved through selection by foraging animals to stand out in a landscape of green foliage. The selection pressures imposed on plants by floral visitors have produced remarkable diversity in floral form, color, and specialized function. As a result, flowers provide more than sensory appeal; they are central to some of the longest recognized and most compelling examples of natural selection and speciation (e.g., Darwin, 1862; Ehrlich and Raven, 1964; Antonovics et al., 1971; Schemske and Bradshaw, 1999; Gardner and Macnair, 2000; Hodges et al., 2002). In the *Origin of Species* (1859), Darwin uses a floral example to describe the process of natural selection:

“Let us now suppose a little sweet juice or nectar [is] to be excreted by the inner bases of the petals of a flower. In this case insects in seeking the nectar would get dusted with pollen, and would certainly often transport the pollen from one flower to the stigma of another flower. [...] Those individual flowers which had the largest glands or nectaries, and which excreted most nectar, would oftenest be visited by insects, and would oftenest be crossed; and so in the long run [plants possessing those flowers] would gain the upper hand” (p. 91).

In this statement, Darwin illustrates how nectar-feeding pollinators impose strong selection on nectar production, promoting evolutionary change. Other floral traits, such as corolla color or shape, may similarly experience selection by pollinators or other floral visitors. Pollinator-
mediated selection on floral phenotypes has been documented in detail for a few, namely temperate, plant species including *Ipomopsis aggregata* (e.g., Campbell, 1989, 1996; Mitchell, 1993; Melendez-Ackerman and Campbell, 1998), *Polemonium viscosum* (e.g., Galen, 1989, 1996, 1999; Galen and Stanton, 1989), *Raphanus sativus* (e.g., Stanton et al., 1986; Stanton, 1987; Strauss et al., 2004), *Dalechampia* spp. (e.g., Armbruster, 1997; Armbruster et al., 2005), *Aquilegia* spp. (e.g., Hodges and Arnold, 1994; Hodges et al., 2002), and *Mimulus* spp. (e.g., Schemske and Bradshaw, 1999). This sampling represents only a fraction of extant diversity in floral phenotypes, and contains very few tropical species or species that separate sexual function within or among flowers. For these and other plant species, the ecological and evolutionary processes important in floral trait origin and maintenance are largely undescribed and untested.

This dissertation examines two poorly understood modifications of floral phenotype, one involving nectar production and the other, calyx morphology. For both, I seek to explain their adaptive significance using current-day interactions between plants and their floral visitors. I use theory and experimentation to explore the trait of gender-biased nectar production, or differential production during the male versus female phase of dichogamous flowers (i.e., hermaphroditic flowers that separate sexual functions through time). I describe and test two key hypotheses for male-biased nectar production: sexual selection and inbreeding avoidance. I also test the adaptive function of the water calyx, a liquid-filled, cup-like structure resulting from the fusion of sepals, which may reduce floral herbivory by submerging buds during development.

This dissertation is unified by two themes, one theoretical and the other botanical. First, male-biased nectar production and water calyces are unified conceptually as traits subject to selection by floral visitors, including both mutualists and enemies. Second, both traits occur in flowers of the same Neotropical herb, *Chrysothemis friedrichsthaliana* (Hanst.) H. E. Moore (Gesneriaceae; Figure 1.1). The reproductive biology and natural history of *C.*
*Chrysothemis friedrichsthaliana* has never before been studied from an evolutionary perspective, and published descriptions of its biology are limited. Skutch (1992) published some basic natural history observations of *C. friedrichsthaliana* in San Isidro de El General, San José Province, Costa Rica. In the only other published study of *C. friedrichsthaliana*, Lu and Mesler (1981) demonstrated that ants disperse its seeds. Detailed data on the phenology, reproductive biology and most important floral visitors of *C. friedrichsthaliana* are absent from published studies, yet are essential to my dissertation. I provide these basic descriptive data here. I also present the results of two experiments that measure nectar in *C. friedrichsthaliana*. These data justify experiments and hypotheses of subsequent chapters. The first data set depicts population-level nectar availability at different times of day. The second demonstrates that male-biased nectar production is genetically-based and persists under different environmental conditions, thereby meeting an important assumption of this dissertation. I conclude the introduction chapter with a brief description of the dissertation structure.

**STUDY ORGANISM**

*Chrysothemis friedrichsthaliana* is a low-growing herbaceous perennial (Figure 1.1) distributed in lowlands throughout Nicaragua, Costa Rica, Panama, Colombia and Ecuador (Skog, 1978; Figure 1.2). It grows terrestrially or epiphytically along streams, forests, and clearings of lowland tropical wet forests (typically less than 200 m elevation). My studies of *C. friedrichsthaliana* use the population at Centro Tropical of the Fundación Neotrópica, in the Golfo Dulce Forestal Reserve (8°42’N, 83°31’W) on Osa Peninsula, Puntarenas Province, Costa
Figure 1.1 *Chrysothemis friedrichsthaliana* habit. Line drawing by Jane E. Carlson.
Figure 1.2 Location of the *Chrysothemis friedrichsthaliana* study population at Centro Tropical of the Fundacion Neotrópica (empty star), in the Golfo Dulce Forestal Reserve, on Osa Peninsula, Puntarenas Province, Costa Rica. Supplementary observations on *C. friedrichsthaliana* in Costa Rica were performed at La Selva Biological Station, Heredia Province (filled star on inset), and at Los Patos Ranger Station, Corcovado National Park, Puntarenas Province (filled star on main map). Black squares on inset represent collection records spanning the range of *C. friedrichsthaliana*, taken from the Missouri Botanical Garden’s VAST (Vascular Tropicos) nomenclature database and associated authority files (VAST, 2007).

More than 1000 reproductive individuals occur at this site, growing in dense, nearly continuous patches along the edges of secondary rain forest and pasture. I made supplementary observations on three additional *C. friedrichsthaliana* populations located ~1 km west of Centro Tropical, at La Selva Biological Research Station (10°26' N, 83°59' W) in Heredia Province, and at Los Patos Ranger Station (8°34'N 083°31'W) in Corcovado National Park, Puntarenas Province (Figure 1.2).
Phenology and Reproductive Biology

The most important phenological events in *C. friedrichsthaliana* are associated with seasonal increases in precipitation. Plants spend the dry season (January - March) as underground tubers or dormant seeds (Skutch, 1992). Vegetative growth begins in April, the start of the wet season, and ends in November, the end of the wet season. Flower production begins in May and continues through November (Figure 1.3). Flowers are protandrous and last two days, functioning as males on the first day and females on the second. If ovules are fertilized, the flower develops a single capsule, which matures over a 5-week period (mean capsule development time = 36.5 d, SD = 2.46, *N* = 167). The capsule dehisces to expose hundreds of tiny, ant-dispersed seeds, each with an eliasome. Near the end of the rainy season, aboveground vegetative parts die back. Plants and ungerminated seeds remain dormant until the following wet season (Skutch, 1992; J. E. Carlson, unpublished data).

**Figure 1.3** Daily flower production rate of *Chrysothemis friedrichsthaliana* during three consecutive flowering seasons. For at least 99 days between mid-June and mid-October 2004-2005, I counted the number of open male-phase and female-phase flowers on each of 99 plants. In 2006, I did so on 40 plants for 39 days. Points mark the first day of a 7-d week, over which all days with data were averaged (*N* = 2-7). Error bars represent ± 1 SE.
*Chrysothemis friedrichsthaliana* plants typically produce less than one new flower per day for most of the flowering season (Figure 1.3). The average rate of flower production was 0.47 per day in 2004 and 0.63 per day in 2005. Production rates varied over both seasons, however. Flower production peaked on August 10 in 2004, at 0.95 flower$\times$plant$^{-1}\times$day$^{-1}$, and on June 17 in 2005 at 1.11 flower$\times$plant$^{-1}\times$day$^{-1}$. Despite low rates of flower production, *C. friedrichsthaliana* plants often had multi-flowered and bisexual plant displays because flowers last 2-3 days and production rates are variable (Figure 1.4). Floral displays with one flower had at least one additional flower of the opposite gender 29% of the time in 2004 and 33% of the time in 2005. Flower loss between phases resulted in a weak population level sex-bias toward male-phase flowers (male-female difference of 0.06 ± 0.097 flower$\times$plant$^{-1}\times$day$^{-1}$; Figure 1.4).

*Chrysothemis friedrichsthaliana* flowers experience slight morphological changes over their 2-3 d lifetimes. Upon anthesis, corollas are 2.56 cm long (SD = 0.13, N = 49), tubular, orange with red nectar guides and are subtended by an enlarged, light-green, cup-like calyx.

![Graph](image)

**Figure 1.4** Display types of *Chrysothemis friedrichsthaliana* plants in 2004 and 2005. Percentages represent the display types of 99 plants, averaged over at least 99 days between mid-June and mid-October for both years. Error bars represent ± 1 SE.
(Figures 1.1 and 1.5; see also Figure 4.1). These features remain relatively constant until the corolla abscises at the end of the female phase. Floral appearance changes between phases because anthers and stigmas occupy different locations (Figure 1.5). Anthers are at the mouth of the corolla during the male phase, and are replaced by the stigma during the female phase.

Flowers are functionally male from ~0530 to ~1730 of the first day. The female phase begins at ~0530 of the second day, but its duration depends on the occurrence of cross-pollination events (Figure 1.6). Naturally visited and experimentally outcrossed female-phase flowers have similar longevity, lasting roughly 12 daylight hours. If female-phase flowers are not outcrossed, however, they can persist in the female phase for up to 36 h. An unvisited female-phase flower capable only of self fertilization (i.e., bagged) is 12 times more likely to delay senescence until after sunrise on the third morning, relative to an experimentally outcrossed female-phase flower ($F_{1, 51} = 6.72, P = 0.012$).

![Figure 1.5](image)

**Figure 1.5** Front-on view of male-phase (left) and female-phase (right) flowers of *Chrysothemis friedrichsthaliana*. Morphological differences are limited to the relative positions of anthers (on left: paired and central; on right: curled back into corolla) and stigma (on left: unseen; on right: central). Line drawings by Jane E. Carlson.
Figure 1.6 Longevity of female-phase *Chrysothemis friedrichsthaliana* flowers under different pollination treatments. Female-phase flowers that were hand-outcrossed then bagged (Outcrossed) had significantly shorter lifespans than those that were bagged without receiving pollen (Control; see text). Experimental procedures were as follows: On 13 different days in Sept. 2005, I applied outcrossed and control treatments to plants with two unvisited female-phase flowers at 0 h after sunrise (i.e. 0530 hours, local time), and I inspected flowers nearly every daylight hour until the corolla detached. I simultaneously monitored an equal number of female-phase flowers exposed to natural visit rates (Natural visit rates; \( N = 52 \) flowers for control and outcrossed; \( N = 124 \) for natural visit rates). I used a binomial logistic regression to compare the probability that an individual flower would delay senescence until the third day in the outcrossed versus control treatment, with source plant as a random effect (Glimmix procedure, SAS 9.1.3).

**Pollinators and Enemies**

*Chrysothemis friedrichsthaliana* is presumed to be hummingbird pollinated (Lu and Mesler, 1981), but this has never been verified. Of the 12+ hummingbird species that occur at Centro Tropical of the Fundacion Neotrópica, only the stripe-throated hermit, *Phaethornis striigularis saturatus* Ridgeway (Trochilidae: Phaethornithinae; Figure 4.1), is a frequent visitor to *C. friedrichsthaliana* plants (Table 1.1). Over two flowering seasons, I videorecorded 718 visits by this species to *C. friedrichsthaliana* flowers, and I videorecorded only four visits
by all other hummingbird species combined. Thus, *P. striigularis* is the only important pollinator of *C. friedrichsthaliana* at the study site. From mid-August to November of 2004 and 2005, *P. striigularis* visited *C. friedrichsthaliana* flowers 1.6 times hour\(^{-1}\)×plant\(^{-1}\), although it made fewer than 0.2 visits hour\(^{-1}\)×plant\(^{-1}\) from June to mid-August of both years (Chapter 3).

*Phaethornis striigularis* is a non-territorial, traplining hummingbird species of low elevation (<1800 m) forested and disturbed habitats of Central and northern South America (Hinkelmann and Schuchmann, 1997). This species is quite common at Centro Tropical, as indicated by its relatively high capture rate in mist nets (Table 1.1). Seventeen individuals were captured and marked with acrylic paints in 2004 and 16 in 2005. Even so, many individuals observed in the population were still unmarked at the end of the mist-netting period. Fewer than five were seen on a regular basis within each year, and one recapture was made in the first year (marks did not last for more than one flowering season). Most re-sightings took place late in the flowering season (August-November), possibly because visit rates were greatly elevated at that time of year.

*Chrysothemis friedrichsthaliana* is likely dependent on *Phaethornis striigularis* for pollination services at multiple sites in Costa Rica. Between August 2003 and October 2006, I observed *P. striigularis* visit *C. friedrichsthaliana* plants in three additional locations: a population ~1 km from Centro Tropical, Los Patos Ranger Station, Corcovado National Park, and La Selva Biological Station (Figure 1.2). No other pollinators were observed visiting *C. friedrichsthaliana* flowers at any of these locations. Given that the geographic range of *P. striigularis* entirely overlaps that of *C. friedrichsthaliana* (Hinkelmann and Schuchmann, 1997; Skog 1978), it is likely that *P. striigularis* is the primary pollinator of this species. It is worth
Table 1.1 Potential and actual hummingbird pollinators of *Chrysothemis friedrichsthaliana* at the Centro Tropical of the Fundación Neotrópica, in the Golfo Dulce Forestal Reserve, on Osa Peninsula, Puntarenas Province, Costa Rica. Birds were caught in nets strung close to the ground near *C. friedrichsthaliana* plants, to selectively target floral visitors. Nets were relocated to a new flower patch every three days.

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<th>Number of recorded visits to <em>Chrysothemis friedrichsthaliana</em>(^b)</th>
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</tr>
<tr>
<td><em>Thalurania colombica</em></td>
<td>Crowned woodnymph</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td><em>Eutoxeres aquila</em></td>
<td>White-tipped sicklebill</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td><em>Florisuga mellivora</em></td>
<td>White-necked Jacobin</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td><em>Klais guimeti</em></td>
<td>Violet-headed hummingbird</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>Heliothyx barroti</em></td>
<td>Purple-crowned fairy</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>Heliomaster longirostris</em></td>
<td>Long-billed starthroat</td>
<td>seen but not caught</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\) Based on 82 days of mist netting (9-12 hrs per day using two 6-m nets of 24mm mesh size) near *C. friedrichsthaliana* plants Jun 26 - Aug 10, 2004 and June 19 - July 26, 2005.

\(^b\) Based on 147 4-10 h days of video observations June 25 - October 13, 2004, and June 13 - October 20, 2005 (except Aug 25 - Sept 8 and Sept 13 - 23). For all species other than *Phaethornis striigularis*, based on all video data as well as casual observations made between June and October 2004 and 2005.

\(^c\) For *P. striigularis*, each capture was of a different individual within a year (17 in 2004 and 16 in 2005), based on acrylic marks on the heads of birds. Marks lasted no more than a single flowering season.
noting, however, that Skutch’s (1992) observations of a *C. friedrichsthaliana* population near San Isidro de El General did not include *P. striigularis*, nor any other major pollinating visitor. Even in the absence of *P. striigularis* or other potential pollinators, my observations indicate that plants can still produce some seeds through self-fertilization (Chapter 3).

Ants are also frequent visitors to the flowers of *C. friedrichsthaliana* (J. E. Carlson, personal observation, Skutch, 1992). Plants are visited by two apparently distinct ant assemblages: those that collect seeds and eliasomes from dehiscent capsules (see Lu and Mesler, 1981), and those that visits flowers to feed on nectar without carrying pollen (i.e., nectar thieves *as per* Inouye, 1983; J. E. Carlson, personal observation). Nectar-thieving ants visit flowers of both phases, and their rates of arrival are similar to each. Roughly 2% of uninfested flowers become ant-infested per daylight hour (based on 10 d of hourly monitoring on 24-38 flowers per day). Once ants arrive, they tend to stay in and around the flower until corolla abscission, or at least for several hours. Since female-phase flowers are older, they are more often ant-infested. By 1700 hours, ants are present in 47% of female-phase and 26% of male-phase flowers.

Despite high visit rates, ants do not appear to affect plant reproduction, in contrast to what has been observed in some other plant species (e.g., Galen, 1999; Irwin et al., 2001). Ants typically do not damage floral tissues while visiting *C. friedrichsthaliana* flowers (J. E. Carlson, personal observation). Furthermore, experimental manipulation of ant presence in flowers showed that *P. striigularis* did not make fewer or shorter visits to female-phase flowers with ants (split-plot ANOVA on number of visits: $F_{1,19} = 0.27, P = 0.61$; on single-visit duration: $F_{1,19} = 0.68, P = 0.42$; based on 20 randomly selected ant/no-ant flower pairs observed for 4 h each).

These findings are applicable to the current debate regarding the consequences of nectar robbers and thieves for plant fitness (e.g., Maloof and Inouye, 2000; Irwin et al., 2001; Irwin and Maloof, 2002). It appears that the nectar-thieving ants of *C. friedrichsthaliana* do not reduce plant
fitness, thus providing no selective advantage to floral traits that may reduce their access into flowers (see also Chapter 2).

The stingless bee *Trigona fulviventris* Guerin-Melenville (Apidae: Meliponinae) is an important antagonist of *C. friedrichsthaliana* flowers (Skutch, 1992; J. E. Carlson, personal observation). This species collects pollen from male-phase flowers, but cannot reach the flower’s nectar. Thus, nearly all *T. fulviventris* visits are to male-phase flowers, since female-phase flowers do not offer pollen. *Trigona fulviventris* typically chews a hole in the corolla at the level of the anthers to remove pollen, or occasionally, it removes entire anthers. Pollen robbing by *T. fulviventris* generally occurs after 0800 hours, and bees may reach over 50% of flowers by the day’s end (based on 10 d of hourly inspections of 24-38 flowers per day).

In addition to nectar and pollen thieves, flowers are also visited by floral herbivores (Chapter 5). Grasshoppers and katydids may consume entire flowers, including calyces. A microlepidopteran moth (Alucitidae) oviposits in unopened buds, and the developing larvae consume anthers and stigmas, but not the corolla (Chapter 5).

**Supplementary Studies of Nectar**

**Standing Crop**

Nectar may be measured in bagged flowers, to estimate true production rates, or in flowers open to pollinator visits, to estimate standing crop. When pollinators are excluded, *C. friedrichsthaliana* flowers produce at least 65% more nectar during the male than the female phase (Chapter 3). I use bagged flowers to estimate male-bias in nectar production because standing crop measures are subject to the vagaries of pollinator visits. Standing crop may nevertheless be useful for interpreting pollinator behavior because it reflects nectar availability as it is encountered by visitors. Below, I describe *C. friedrichsthaliana’s* standing crop in the study population at different times of day and throughout the flowering season.
Methods—On 11 days during the early flowering season (May to mid-August) and 12 days during the late flowering season (mid-August to November) of 2005, I measured standing crop at 0600, 0800, 1000, 1200, and 1400 hours. At each time period, I removed five male- and five female-phase flowers from randomly chosen plants, and I immediately measured nectar with 10-µL calibrated microcapillary tubes and calipers. I measured sugar concentration with a portable sugar/brix refractometer (range 0-32%, accuracy ± 0.2%, Sper Scientific, Scottsdale, Arizona, USA) and used both volume and concentration to obtain mass of sucrose equivalents, or mass of sugar (Kearns and Inouye, 1993). To statistically compare standing crop of nectar (mg sugar) throughout the day and flowering season in both male- and female-phase flowers, I performed a 3-way ANOVA using the Mixed procedure in SAS 9.1.3 (SAS Institute, Cary, North Carolina, USA). Fixed effects were floral gender, time of day and early versus late flowering season.

Results and Discussion—Standing crop in *C. friedrichsthaliana* flowers varied strongly between portions of the flowering season and moderately throughout the day (Figure 1.7A, B). In the early season, most flowers in the population contained some nectar, with a daily average of 1.18 ± 0.04 (mean ± SE) mg sugar × flower⁻¹ (Figure 1.7A). In the late season, however, average standing crop was reduced by more than 6-fold, down to 0.18 ± 0.04 mg sugar × flower⁻¹ (Figure 1.7B). The seasonal reduction in standing crop was also observed daily, at a much reduced scale. Slightly more nectar was available in flowers of both phases in the morning than in the afternoon (Figure 1.7 A,B). Although standing crop exhibited significant fluctuations daily and seasonally, it was always similar between floral phases (Table 1.2). Thus, unlike true production rates, standing crop was not male-biased.
The observed patterns in *C. friedrichsthaliana* standing crop can be explained using rates of nectar production and pollinator visits. As I demonstrate in Chapter 3, visit rates by *P. striigularis* are 8 times higher in the late relative to early season. Standing crop appropriately reflects this increase, as it is significantly lower in the late season. Furthermore, nectar production and pollinator visit rates decline throughout the day (Chapter 3), which could explain why standing crop decreases over the course of the day, as long as production decreases more than visits. Finally, *P. striigularis* makes 86% more visits to male-phase flowers (Chapter 3). This increased frequency of male-phase visits may explain why the roughly 65% male-bias in nectar production is not present in standing crop measurements.

Some investigators argue that standing crop provides a superior depiction of available nectar resources as compared to nectar production rates (e.g., Zimmerman, 1988). If pollinators preferentially use standing crop to shape foraging decisions, they may not perceive nectar
Table 1.2 Results of three-way ANOVA of nectar standing crop (mg sugar) of *Chrysothemis friedrichsthaliana* (see also Figure 1.7). Time of day levels were 0600, 0800, 1000, 1200, 1400, and 1600 hours. Season levels were early and late. df = numerator degrees of freedom, denominator degrees of freedom.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Season</td>
<td>1, 1209</td>
<td>338.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Time of day</td>
<td>5, 1029</td>
<td>5.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Time of day × season</td>
<td>5, 1209</td>
<td>4.9</td>
<td>0.0002</td>
</tr>
<tr>
<td>Gender</td>
<td>1, 1029</td>
<td>0.1</td>
<td>0.76</td>
</tr>
<tr>
<td>Time of day × gender</td>
<td>5, 1029</td>
<td>0.3</td>
<td>0.93</td>
</tr>
<tr>
<td>Gender × season</td>
<td>1, 1209</td>
<td>0.2</td>
<td>0.67</td>
</tr>
<tr>
<td>Time of day × gender × season</td>
<td>5, 1209</td>
<td>0.4</td>
<td>0.85</td>
</tr>
</tbody>
</table>

production biases in plants like *C. friedrichsthaliana*. This argument is not particularly relevant for *C. friedrichsthaliana*, however, because *P. striigularis* is unlikely to rely solely on the nectar contents in a flower at the moment it is visited. *Phaethornis striigularis* hummingbirds visually distinguish male-and female-phase flowers, and they base decisions on both morphology and nectar rewards (Chapter 4). Thus, standing crop measures do not provide meaningful estimates of gender-biased nectar rewards in *C. friedrichsthaliana*. They simply depict the dynamics of nectar production and depletion by pollinators.

**Environmental Versus Genetic Influence on Nectar**

Seasonal changes in rainfall and insolation on the Osa Peninsula of Costa Rica do not obscure or reverse population-wide patterns of male-biased nectar production in *C. friedrichsthaliana* (Chapter 3). This suggests that male-biased nectar production in *C. friedrichsthaliana* has a genetic basis. Evidence presented in Chapter 3 is, nevertheless, based on field observations rather than manipulative experiments. Here, I provide direct evidence for genetic variation in nectar production rates, and I demonstrate limited phenotypic plasticity in response to light availability. I do so using measurements of male- and female-phase nectar
production from clonally propagated and potted *C. friedrichsthaliana* cuttings, which were placed in greenhouses with low or high light. Similar methods have been used by others to measure heritability of nectar production rates and genotype × environment interactions (e.g., Hodges, 1993; Boose, 1997).

**Methods**—In late May 2005, I collected 11 large plants from different locations with similar light availability on the property of Centro Tropical of the Fundación Neotrópica. I divided each plant (genet) into two medium-sized cuttings (ramets), and I potted one ramet in a shaded greenhouse, covered in clear plastic and 60% shade cloth, and the other in an adjacent greenhouse, covered in clear plastic under full sun. All plants received water daily, in equal amounts approximating natural rainfall, and they were randomly relocated within greenhouses twice a week. Plants under both low and high light began producing new flowers approximately two months after potting. The first flower each plant produced was measured during the male phase, and the second, the female phase. I alternated between phases within each plant until the experiment’s end, in mid-October 2005. To measure nectar replenishment rates, I drained and bagged selected flowers at 1000 hours. At 1200 hours, I collected flowers and measured their nectar volumes and sucrose concentrations, using the same methods as for standing crop. I tested the effects of genet, gender, light availability and interactions using a 3-way ANOVA on mass of sucrose equivalents (Mixed Procedure, SAS 9.1.3).

**Results and Discussion**—Nectar measurements from clonally propagated ramets suggest that variability in male-biased nectar production has both a genetic and environmental basis (Table 1.3). Differences in nectar replenishment rates among *C. friedrichsthaliana* flowers were best explained by a flower’s gender, followed by the source genet, and finally, by the light environment. Male-phase nectar replenishment rate was roughly three times higher than that of the female-phase, averaged over plants and light treatments (Figure 1.8). There was significant
Table 1.3 Results of three-way ANOVA of 2-h nectar replenishment rate (mg sugar) of 11 clonally propagated *Chrysothemis friedrichsthaliana* plants grown under low versus high light (see also Figure 1.8). Ramets in the high light treatment were kept in a greenhouse under full sun for the 5-month study (June-October 2005), and those in the low light treatment were kept in an adjacent greenhouse covered in 60% shade cloth. Male- and female-phase nectar replenishment rate was measured daily at 1200 hours, following a 2-h replenishment interval.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>1, 468</td>
<td>80.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Source plant</td>
<td>10, 468</td>
<td>5.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gender × source plant</td>
<td>10, 468</td>
<td>0.7</td>
<td>0.75</td>
</tr>
<tr>
<td>Light treatment</td>
<td>1, 468</td>
<td>4.3</td>
<td>0.04</td>
</tr>
<tr>
<td>Gender × light treatment</td>
<td>1, 468</td>
<td>0.1</td>
<td>0.79</td>
</tr>
<tr>
<td>Source plant × light treatment</td>
<td>10, 468</td>
<td>1.5</td>
<td>0.12</td>
</tr>
<tr>
<td>Source plant × light treatment × gender</td>
<td>10, 468</td>
<td>1.4</td>
<td>0.16</td>
</tr>
</tbody>
</table>

genetic variation in nectar production rates among plants, but despite variability, rewards were consistently male-biased (i.e., gender × source plant interaction non-significant; Table 1.3).

Variation in nectar production that could be attributed to genotype was not specific to a light treatment. Finally, plants in a high light environment produced slightly less nectar than those in a low light environment, regardless of floral phase or genotype (Table 1.3). In sum, the effect of genotype was much stronger than that of light environment, and there was no genotype × environment interaction. This suggests that under the range of experimental conditions I imposed, phenotypic plasticity in nectar production is relatively low, and differences among genotypes are relatively strong. A few details regarding the current study nevertheless warrant further explanation.

Nectar replenishment rates in potted *C. friedrichsthaliana* plants were considerably lower than observed in the wild. The 2-h nectar replenishment rates of naturally-growing plants in
August – September 2005 were 56-83% higher during the male phase, and 26-117% higher during the female phase, as compared to flowers of either phase from potted plants (August-Sept 2005 nectar replenishment rate on naturally growing plants; male-phase: 0.30 ± 0.03; female-phase: 0.085 ± 0.034, see also Chapter 3). Potted plants may have produced less nectar overall because they were water-limited (e.g., Boose, 1997). Although I watered experimental plants to mimic natural rainfall, air temperatures were higher in the greenhouses, causing evapotranspiration rates to be higher as well. The possibility that potted plants were water-limited is also corroborated by experimental results. Specifically, plants in the cooler, shaded greenhouse produced more nectar than plants in the hotter, unshaded greenhouse (Figure 1.8). My observations suggest that many physiological processes of *C. friedrichsthaliana*, including nectar production, are affected by water availability on a daily basis. Even so, the most probable
consequences of environmental plasticity with water availability are changes in net nectar production, rather than a reverse in male-biased nectar production.

Although my experimental design was appropriate to test for genotype and environmental effects on nectar, interpretations must be made with some caution. For example, if nectar production rates are environmentally determined during early developmental stages (e.g., before plants are clonally propagated), then my design would erroneously interpret source plant effects as genotype effects. Had I self-fertilized plants and grown full-siblings in low versus high light environments, I may have been able to account for this possibility (e.g., Mitchell and Shaw, 1993). Instead, I partially accounted for developmental effects by collecting all 11 genets from very similar light environments, less than 100 m apart. Assuming I was able to account for developmental effects, these results provide a solid foundation for tests of natural and sexual selection on nectar production, which I describe in Chapter 2, and test in Chapters 3 and 4.

**DISSERTATION STRUCTURE**

As the preceding sections show, the biology and natural history of *C. friedrichsthaliana* make it an appropriate candidate for tests of natural and sexual selection on floral traits. The remainder of my dissertation is devoted to tests of evolutionary hypotheses for floral trait functions in *C. friedrichsthaliana*, using predictions from theory and field-collected ecological data. Below, I briefly describe each dissertation chapter.

In Chapter 2, I summarize patterns of gender-biased nectar production from the literature and critically review theories relevant to its evolution. I list and describe 41 species with gender-biased nectar production, and I consider evolutionary hypotheses based on sexual selection, natural selection for inbreeding avoidance, resource trade-offs, enemies, and pleiotropic effects. This chapter is published in The Botanical Review (henceforth Carlson and Harms, 2006).
In Chapter 3, I narrow my focus from gender-biased nectar production in dichogamous plants to male-biased nectar production in *C. friedrichsthaliana*. I describe a series of observations on *C. friedrichsthaliana* and *P. striigularis* that test predictions of sexual selection theory. I present measurements of male-biased nectar production, pollinator visit rates, and male versus female plant fecundity, and I compare each with specific sexual selection predictions. This chapter is published in the American Journal of Botany (henceforth Carlson, 2007).

In Chapter 4, I build on the results from Chapter 3 to comprehensively test the sexual selection and inbreeding avoidance hypotheses in *C. friedrichsthaliana*. I present the results of nectar manipulations in real and artificial flowers, which demonstrate the nectar-based foraging preferences of *P. striigularis*. I also assess the maternal cost of inbreeding based on hand-pollination experiments. I then compare these results to a final prediction of the sexual selection hypothesis and two predictions of the inbreeding avoidance hypothesis.

In Chapter 5, I use the water calyx of *C. friedrichsthaliana* to test the hypothesis that sepal morphology is largely influenced by selection by floral herbivores. First, I describe how buds are frequently attacked by ovipositing moths (Alucitidae), and how anthers and stigmas are consumed by their larvae before corollas open. Second, I test whether alucitid egg deposition and subsequent herbivory are reduced in buds covered by liquid, relative to buds in calyces with liquid experimentally removed. This chapter is published in Biology Letters (henceforth, Carlson and Harms, 2007).

In Chapter 6, I conclude with a brief overview of my results. I also propose future directions for research, namely using *Chrysothemis* congeners and species from Table 2.1.

**LITERATURE CITED**


——. 1862. On the various contrivances by which British and foreign orchids are fertilised by insects. John Murray, London.


Chapter 2

The Evolution of Gender-Biased Nectar Production in Hermaphroditic Plants

INTRODUCTION

Gender-specific traits in plants with simultaneously bisexual flowers may appear to be confined to the primary sexual structures of the flower. For example, traits of the pollen grain are generally unambiguously male-specific in function, whereas the nectar production rate or the corolla color, both secondary sexual traits, cannot be immediately associated with either male or female floral function. Secondary sexual traits of flowers can, nonetheless, contribute disproportionately to male or female reproductive output. Corolla morphology and nectar volume, for example, have been shown to serve gender-biased functions in a few well-studied species (e.g., Bell, 1985; Stanton et al., 1986; Campbell, 1989; Galen and Stanton, 1989; Melendez-Ackerman and Campbell, 1998). Nonetheless, the co-occurrence of both primary and secondary sexual structures within the simultaneously bisexual flower introduces some ambiguity into studies of the gender-biased function of secondary traits, because most secondary traits are physically associated with both floral genders.

Dichogamous plant species—those that temporally separate male function and female function within flowers—may provide a unique means of reducing this ambiguity. In dichogamous species, the expression of a secondary sexual trait may vary with sexual phase. Floral nectar production rates, for example, can be higher during one of the sexual phases, and the result is gender-biased nectar production (GBNP). The unequal distribution of nectar between male and female phases immediately associates a different nectar production schedule.

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with each gender and may reflect a true functional bias towards male or female fitness. Gender-biased nectar production is therefore likely to be particularly informative about the nature of sexual or natural selection on secondary traits, yet it has been studied only in a few isolated cases.

In this paper, we show that a diverse group of hermaphroditic plants exhibits gender-biased nectar production, and we critically evaluate hypotheses that may explain the nectar production patterns in these species. First, we summarize published examples of dichogamous species that exhibit nectar production biases, and we describe trends apparent among these species. Then, we explore theories relevant to the trait’s evolution, which correspond to five main categories: sexual selection, natural selection against inbreeding, resource tradeoffs, floral enemy effects and evolution by correlated traits (pleiotropy). We focus our discussion on sexual selection and inbreeding avoidance theories, and from these, we extract four main hypotheses that may explain gender-biased nectar production. We then discuss the remaining three topics and finish with an experimental framework designed to help researchers discriminate among the main hypotheses.

**GENDER-BIASED NECTAR PRODUCTION (GBNP) IN DICHOGAMOUS PLANTS**

Dichogamy is remarkably widespread within the angiosperms (Bertin and Newman, 1993; Barrett, 2003). Within dichogamous species, the number that biases nectar production towards the male or female phase is currently unknown. We attempted to compile an exhaustive list of hermaphroditic species with gender-biased nectar production from the literature. Topics of searched literature included, but were not limited to, the physiology and biology of nectar production, pollinator behavior at inflorescences, and sexual selection in hermaphroditic plants. Plant species were included if dichogamy was clearly established, if nectar production rates in
male-phase and female-phase flowers were consistently different (i.e., one phase was almost always more rewarding), and if all plants in the study populations produced only bisexual flowers (i.e., studies of monoecious, dioecious and gynodioecious plants were excluded).

Results from Literature Search

Commonalities among Dichogamous Plants with GBNP

Our literature search revealed 41 dichogamous species with male- or female-biased nectar production, representing 18 families and 22 genera (Table 2.1). Despite wide taxonomic diversity among species, nearly all share a few key characteristics. Most genera (20 of 22) are protandrous (male phase precedes female phase in time); the only two protogynous (female phase precedes male phase) taxa are *Ribes speciosum* and *Scrophularia* spp. All listed species may have more than one flower open at the same time, although flower counts range from less than five (*Campanula rotundifolia*; Cresswell and Robertson, 1994) to greater than 100 (*Agave mckelvyana*; Sutherland, 1987). Further, flowers of all but four species develop asynchronously, such that individual plants may have both male- and female-phase flowers open simultaneously. As a result, if self-compatible, these 37 species are susceptible to inbreeding between flowers on the same plant (in addition to inbreeding within flowers). Those species unlikely to be self-pollinated by flowers on the same plant, because of synchronous flower development throughout whole plants, are *Agave mckelvyana, Alstroemeria aurea, Bomarea acutifolia,* and *Pentagonia macrophylla*. Almost three-fourths of the listed species produce flowers in racemes (flowers mature sequentially from the bottom to the top of a vertical inflorescence). This floral arrangement frequently results in a vertical gradient of nectar rewards within the inflorescence, the direction of which depends on the gender-bias and the type of dichogamy (Table 2.1).
Table 2.1 Dichogamous species with gender-biased nectar production

<table>
<thead>
<tr>
<th>Family and species</th>
<th>Nectar collection technique</th>
<th>Gender bias</th>
<th>Volume of nectar produced (μL)</th>
<th>Mass of sugar produced (mg)</th>
<th>M:F ratio (b)</th>
<th>Pollinator type (c)</th>
<th>Vertical nectar gradient (d)</th>
<th>Visit bias (e)</th>
<th>Reference(s)(f)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protandrous species:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Campanulaceae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Campanula rotundifolia</em></td>
<td>Standing</td>
<td>F</td>
<td>0</td>
<td>0.01</td>
<td>0</td>
<td>8.25 × 10(^{-2})</td>
<td>0 : 0.01</td>
<td>B</td>
<td>no</td>
</tr>
<tr>
<td><strong>Lamiaceae</strong></td>
<td>12 hour</td>
<td>F(^g)</td>
<td>0.047</td>
<td>0.093</td>
<td>2.4 × 10(^{-3})</td>
<td>2.6 × 10(^{-3})</td>
<td>1 : 2.0*</td>
<td>B</td>
<td>no</td>
</tr>
<tr>
<td><strong>Brassicaceae</strong></td>
<td>Unknown</td>
<td>F</td>
<td>0</td>
<td>&gt;0</td>
<td>0</td>
<td>&gt;0</td>
<td>0 : &gt;0</td>
<td>I</td>
<td>?</td>
</tr>
<tr>
<td><em>Streptanthus culteri</em></td>
<td>Unknown</td>
<td>F</td>
<td>0</td>
<td>&gt;0</td>
<td>0</td>
<td>&gt;0</td>
<td>0 : &gt;0</td>
<td>I</td>
<td>?</td>
</tr>
<tr>
<td><strong>Plantaginaceae</strong></td>
<td>24 hour</td>
<td>F</td>
<td>less</td>
<td>more</td>
<td>less</td>
<td>more</td>
<td>B</td>
<td>yes (\uparrow)</td>
<td>Y</td>
</tr>
<tr>
<td><em>Digitalis purpurea</em></td>
<td>12 hour</td>
<td>F</td>
<td>—</td>
<td>—</td>
<td>6.3 × 10(^{-4})</td>
<td>11.7 × 10(^{-4})</td>
<td>1 : 1.9*</td>
<td>B</td>
<td>yes (\uparrow)</td>
</tr>
<tr>
<td><strong>Onagraceae</strong></td>
<td>24 hour</td>
<td>F(^h)</td>
<td>1.95</td>
<td>2.62</td>
<td>—</td>
<td>—</td>
<td>1 : 1.3*</td>
<td>B</td>
<td>yes (\uparrow)</td>
</tr>
<tr>
<td><em>Delphinium virescens</em></td>
<td>Standing</td>
<td>F(^h)</td>
<td>0.3</td>
<td>0.45</td>
<td>—</td>
<td>—</td>
<td>1 : 1.5</td>
<td>B</td>
<td>yes (\uparrow)</td>
</tr>
<tr>
<td><em>Delphinium barbeyi</em></td>
<td>24 hour</td>
<td>F(^h)</td>
<td>2.4</td>
<td>4.42</td>
<td>—</td>
<td>—</td>
<td>1 : 1.8*</td>
<td>B</td>
<td>yes (\uparrow)</td>
</tr>
<tr>
<td><em>Delphinium nelsonii</em></td>
<td>Standing</td>
<td>F</td>
<td>—</td>
<td>—</td>
<td>0.21</td>
<td>0.43</td>
<td>1 : 2.0*</td>
<td>B/H</td>
<td>yes (\uparrow)</td>
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<tr>
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<td>24 hour</td>
<td>F</td>
<td>70.7</td>
<td>122.6</td>
<td>—</td>
<td>—</td>
<td>1 : 1.7</td>
<td>H</td>
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<tr>
<td><em>Pentagonia macrophylla</em></td>
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<td>50</td>
<td>25</td>
<td>—</td>
<td>—</td>
<td>2.0 : 1*</td>
<td>H</td>
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<td><em>Macleania bullata</em></td>
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<tr>
<td><strong>Gesneriaceae</strong></td>
<td>12 hour</td>
<td>M</td>
<td>9.28</td>
<td>6.36</td>
<td>3.05</td>
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<td>M</td>
<td>1.76</td>
<td>1.18</td>
<td>—</td>
<td>—</td>
<td>1.5 : 1*</td>
<td>H</td>
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<td>Lifetime</td>
<td>M</td>
<td>15.2</td>
<td>11.77</td>
<td>—</td>
<td>—</td>
<td>1.3 : 1*</td>
<td>H</td>
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</table>

\(^{a}\) Collection technique (standing, 12 hour, 24 hour, lifetime, vertical nectar gradient, pollinator type (B, H, Y)).

\(^{b}\) M:F ratio (male:female) in the table represents the volume of nectar produced.

\(^{c}\) Pollinator type (B: bees, H: hummingbirds, Y: both).

\(^{d}\) Vertical nectar gradient: yes \(\uparrow\) indicates a gradient exists.

\(^{e}\) Visit bias: yes \(\uparrow\) indicates a visit bias exists.

\(^{f}\) Reference(s): Cresswell and Robertson, 1994; Gonzalez et al., 1995; Rollins, 1963; Rollins, 1963; Best and Bierzychudek, 1982; Pyke, 1978b; Galen and Plowright, 1985a, 1985b; Pyke, 1978b; Waddington, 1981; Pyke, 1978b; Pyke, 1978b; Cruden et al., 1983; McDade, 1986; Navarro, 2001; J. E. Carlson, unpublished data; Lara and Ornelas, 2001; Feininger, 1978; Lara and Ornelas, 2002.
<table>
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<tr>
<th>Family and species</th>
<th>Nectar collection technique</th>
<th>Gender bias</th>
<th>Volume of nectar produced (μL)</th>
<th>Mass of sugar produced (mg)</th>
<th>M:F ratio</th>
<th>Pollinator type</th>
<th>Vertical nectar gradient</th>
<th>Visit bias</th>
<th>Reference(s)</th>
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<td>Lifetime</td>
<td>M</td>
<td>1715</td>
<td>1368</td>
<td>117</td>
<td>123</td>
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<td>less</td>
<td>S</td>
<td>?</td>
<td>?</td>
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<td>M</td>
<td>more</td>
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<td>more</td>
<td>less</td>
<td>S</td>
<td>?</td>
<td>?</td>
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<td>—</td>
<td>—</td>
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<td>less</td>
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<td>1.2</td>
<td>1.16</td>
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<td>58%</td>
<td>42%</td>
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<td>1.2</td>
<td>1.4 : 1</td>
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<td>1.16</td>
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<td>0.38</td>
<td>1.2 : 1*</td>
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<td>5.7</td>
<td>8.1</td>
<td>1.8</td>
<td>3.0</td>
<td>1 : 1.4</td>
<td>W</td>
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<td>8.7</td>
<td>11.7</td>
<td>2.6</td>
<td>3.2</td>
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<td>9.1</td>
<td>2.6</td>
<td>3.7</td>
<td>1 : 1.4</td>
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<td>F</td>
<td>10.1</td>
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<td>4.3</td>
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<td>F</td>
<td>22.2</td>
<td>38.2</td>
<td>7.8</td>
<td>11.5</td>
<td>1 : 1.7</td>
<td>W</td>
<td>yes ↓</td>
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Table 2.1 (continued)

<table>
<thead>
<tr>
<th>Family and species</th>
<th>Nectar collection technique&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Gender bias</th>
<th>Volume of nectar produced (μL)</th>
<th>Mass of sugar produced (mg)</th>
<th>M:F ratio&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Pollinator type&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Vertical nectar gradient&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Visit bias&lt;sup&gt;e&lt;/sup&gt;</th>
<th>Reference(s)&lt;sup&gt;f&lt;/sup&gt;</th>
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<td>Scrophularia viciosoi</td>
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<td>F&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.1</td>
<td>9.1</td>
<td>2.6</td>
<td>4.3</td>
<td>1 : 1.5</td>
<td>W</td>
<td>yes ? Oliveira and Alcaraz, 1993</td>
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<td>12 hour</td>
<td>F&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.2</td>
<td>17.0</td>
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<td>6.0</td>
<td>1 : 1.5</td>
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<td>F&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.6</td>
<td>21.9</td>
<td>6.1</td>
<td>7.6</td>
<td>1 : 1.1</td>
<td>W</td>
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<td>F</td>
<td>less</td>
<td>more</td>
<td>less</td>
<td>more</td>
<td>W</td>
<td>no</td>
<td>? Corbet et al., 1981</td>
</tr>
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<td>M&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.2</td>
<td>32</td>
<td>8.9</td>
<td>8.1</td>
<td>1.1 : 1</td>
<td>W</td>
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<td>M&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.2</td>
<td>8.3</td>
<td>3.7</td>
<td>3.3</td>
<td>1.1 : 1</td>
<td>W</td>
<td>yes ? Oliveira and Alcaraz, 1993</td>
</tr>
<tr>
<td>Scrophularia oxyrhyncha</td>
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<td>M&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.9</td>
<td>5.2</td>
<td>2.1</td>
<td>2.0</td>
<td>1.1 : 1</td>
<td>W</td>
<td>yes ? Oliveira and Alcaraz, 1993</td>
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<tr>
<td>Ribes speciosum</td>
<td>12 hour</td>
<td>M</td>
<td>more</td>
<td>less</td>
<td>more</td>
<td>less</td>
<td>H</td>
<td>no</td>
<td>Y Stiles, 1976</td>
</tr>
</tbody>
</table>

<sup>a</sup>Nectar was collected from flowers using the following techniques: 12-hour nectar production rates of bagged flowers (12 hr), 24-hour nectar production rates of bagged flowers (24 hr), Lifetime production for each phase, measured over the entire duration of the male or female phase (Lifetime), Standing crop of unbagged flowers sampled once at a given time of day (Standing), and Unknown measurement technique (Unknown).

<sup>b</sup>The male-to-female nectar production ratio (M:F) was calculated on volume of nectar (μL) unless only mass of sugar was available. When the differences between male- and female-phase nectar production were tested for statistical significance, the M:F ratio is flagged by an asterisk (*).

<sup>c</sup>Pollinator types are Bee (B), Insect (I), Hummingbird (H), Sunbird (S), and Wasp (W).

<sup>d</sup>Arrows denote the direction within the inflorescence in which per-flower nectar production declines. For example, an upward arrow indicates that flowers near the top of the inflorescence produce the most nectar, and those near the bottom of the inflorescence produce the least.

<sup>e</sup>A visit bias was observed when the pollinator more frequently visited flowers of the more-rewarding phase.

<sup>f</sup>When two or more references are listed, a dagger (†) denotes the source(s) of the reported nectar production data.

<sup>g</sup>Volume of nectar in male- and female-phase flowers was significantly different, but mass of sucrose in flower phases was not different. This was most likely because sample sizes were smaller for mass measurements (N = 14 for volume, N = 7 for mass; Gonzalez et al., 1995).

<sup>h</sup>Nectar measurements were extrapolated from published data. Any statistic performed on these species did not use the extrapolated data.
Volume of nectar in male- and female-phase flowers was taken at roughly 22 rather than 24 hours since bagging, because female-phase flowers were wilted and difficult to measure by late afternoon (McDade, 1986).

Volume of nectar produced was consistently male-biased, however mass of sucrose produced was not significantly gender-biased, and concentration was consistently female-biased (Burd, 1992).

Plants studied in New Mexico did not exhibit a significant gender bias (Cruden et al., 1983), but those in Pennsylvania and Mexico did (Devlin and Stephenson, 1985; Lara and Ornelas, 2002; see Langenberger and Davis, 2002).

Lifetime nectar production was male-biased, but standing crop was not gender-biased, possibly because bees more frequently visited male-phase flowers (Aizen and Basilio, 1998).

Percentage of daily total nectar volume produced per branch (Sutherland, 1987).

Mass of sugar produced was consistently male-biased, however volume was not gender-biased (Guitián et al., 1995).

Gradient was present early in the flowering season, but it was likely to disappear later in the season (Olivencia and Alcaraz, 1993).

Visit bias toward phase with more nectar occurred only after all pollen was removed from male-phase flowers (Cresswell and Robertson, 1994).

Visit bias was towards female-phase flowers, the less-rewarding phase (Lara and Ornelas, 2001).
In summary, most listed plants are protandrous, have both male and female flowers open at the same time, and at least half have a nectar gradient within inflorescences.

**GBNP is Male-Biased as Often as Female-Biased**

The nectar production rate is greater in the male phase in 21 species and in the female phase in 20 species. At a broader taxonomic level, 14 genera include species with male biases and nine include species with female biases. Willson and Ågren (1989) noted a similar relative frequency of male- and female-biased nectar production in taxa with unisexual flowers as well. In their literature review, they found a total of 23 monoecious or dioecious species with differential rewards in male and female flowers; 11 species produced more nectar in male flowers and 12 species produced more nectar in female flowers. Nectar production biases in monoeccious, dioecious and gynodiecous plants have been reviewed recently (Eckhart, 1999) and are being actively researched (Ashworth and Galleto, 2002; Gehring et al., 2004).

**Nectar Production Biases are Modest in Most Plants with GBNP**

Species with gender-biased nectar production generally exhibited a less than two-fold difference in nectar production between male- and female-phase flowers, although average nectar production per flower varied widely among species (Table 2.1). The male/female ratio of nectar production ranged from 1.1 to 5.2 for species with male-biased nectar production (mean=1.9, median=1.5), whereas the female/male ratio ranged from 1.2 to 3.6 for species with female-biased nectar production (mean=1.6, median=1.5). These ratios exclude the three protandrous species that produce no nectar during the male phase. Although nectar production ratios may at first appear small, pollinators can often discriminate against less-rewarding flowers on plants with female/male nectar production ratios as low as 1.2 (Table 2.1).
Trends within Male-Biased and Female-Biased Groups and within Taxa

Species that exhibit the same nectar production bias (male or female) tend to have similar pollinators and inflorescence structures, if the genus *Scrophularia* is considered separately. Species with female-biased nectar production are most often bee-pollinated (8 of 11) and produce the most-rewarding flowers (female) at the base of racemose inflorescences and the least-rewarding flowers (male) at the top (6 of 11). Species with male-biased nectar production, in contrast, are typically bird-pollinated (13 of 18) and lack spatial arrangements of nectar rewards or produce the least-rewarding flowers (female) at the top of racemose inflorescences. These comparisons are not phylogenetically independent, and thus, some similarities may be due to common ancestry rather than convergent evolution.

The species in the genus *Scrophularia* generally lack commonalities observed in the larger group. For example, unlike other species with gender-biased nectar production, *Scrophularia* species are protogynous and wasp-pollinated (Olivencia and Alcaraz, 1993). The relatively small M:F nectar production ratios and the lack of individual statistical tests on the nectar production data of some *Scrophularia* species (e.g., the species with male-biases) suggest that a few of the measured biases may be biologically insignificant.

Gender-biased nectar production may be a phylogenetically conserved trait in some taxa, as evidenced in part by its presence in twelve *Scrophularia* species, six *Lobelia* species, three *Delphinium* species, and two *Streptanthus* species. Despite frequent recurrence of gender-biases among congeneres, the direction of a nectar production-bias is not necessarily fixed within lineages. *Scrophularia*, as already mentioned, includes at least nine species with female-biased nectar production and at least three species with male-biased nectar production (Olivencia and Alcaraz, 1993). The direction of bias may also differ among individuals in the same population (*Lobelia deckenii* on Mount Kilimanjaro, Burd, 1992) or among conspecific populations (*Lobelia*...
cardinalis in New Mexico, Cruden et al., 1983; compared to those in Pennsylvania, Devlin and Stephenson, 1985; or in Mexico, Lara and Ornelas, 2002; see Langenberger and Davis, 2002).

**Is GBNP Heritable?**

Discrepancies within species in the direction of nectar production bias may reflect the influence of the environment on a plastic trait (e.g., Southwick and Southwick, 1983; Campbell, 1996), and/or they may reflect genetic variation among individuals. Nectar volume has high genetic variation and is heritable in *Echium vulgare* and at least five other angiosperms (Zimmerman and Pyke, 1986; Hodges, 1993; Mitchell and Shaw, 1993; Boose, 1997; Klinkhamer and van der Veen-van Wijk, 1999; Leiss et al., 2004; reviewed in Mitchell, 2004). The heritabilities of traits causing a temporal change in nectar production rates, the purported targets of selection for this review, are not known for any dichogamous species (Mitchell, 2004). Most likely, nectar production-biases, like nectar production rates, are influenced by both genes and the environment (Boose, 1997; Vogler et al., 1999; Leiss et al., 2004).

We assume that the temporal pattern of nectar production has some heritable basis for most species. In this light, we consider the listed species as valuable experimental units for evolutionary studies of secondary floral traits. Such studies may test basic predictions of the evolved or optimal direction of gender-biases in dichogamous plants, based on sexual selection, inbreeding avoidance, and other theories. In the following sections, we critically review the theoretical background of these hypotheses, and then we consider their utility in light of currently available observations.

**EVOLUTIONARY HYPOTHESES FOR GBNP**

**Sexual Selection Hypotheses**

In both plants and animals, sexual selection results from competition for mates between individuals of the same sex and species. Sexual competition is unique in plants however,
because it can occur indirectly via pollinators (Charnov, 1979; Lloyd and Yates, 1982; Arnold, 1994). In effect, plants ‘court’ the pollinators rather than the opposite sex itself, making pollinators the target audience for many secondary traits of hermaphroditic flowers. Despite clear parallels between animal and plant examples of sexual selection, the analogy of sexual selection across the two groups remains controversial, questioned by some (Broyles and Wyatt, 1990; Wilson et al., 1994; Broyles and Wyatt, 1995) and advocated by others (Stephenson and Bertin, 1983; Andersson, 1994; Arnold, 1994; Willson, 1994; Queller, 1997; Skogsmyr and Lankinen, 2002).

Selection that acts predominately through one floral gender is the subject of a growing body of theory within floral evolution, yet some recent contributors make little mention of sexual selection and, in some instances, use instead the term gender-specific selection (e.g., Burd and Callahan, 2000; Ashman and Morgan, 2004). Gender-specific selection is like sexual selection in that it involves different effects on male and female fitness, but unlike sexual selection, these differences need not be caused by intra-sexual competition for mates (i.e., they may be caused by natural selection). Use of the term gender-specific selection circumvents the need to further classify floral traits as naturally or sexually selected, a task that may be both controversial and difficult (Lloyd and Yates, 1982; Skogsmyr and Lankinen, 2002). Nonetheless, sexual selection has almost certainly played a distinctive role in the evolution and maintenance of many floral traits (Willson, 1994; Skogsmyr and Lankinen, 2002), and we advocate its explicit consideration in studies of floral evolution.

Gender-biased expression of nectar in hermaphrodites likely indicates a history of sexual selection for many species. Here we consider two separate sexual selection hypotheses for the function of this trait. The first, called the male function hypothesis, predicts pollinator limitation on male fitness as the cause of sexual selection for predominately male-functioning traits
The male function hypothesis has been defined similarly by others, although without explicit association with sexual selection (Burd and Callahan, 2000; Ashman and Morgan, 2004). This hypothesis has also been called the pollen donation hypothesis (Broyles and Wyatt, 1990), or the fleurs-du-mâle hypothesis (Queller, 1983). The second hypothesis predicts pollen limitation on female fitness as the cause of selection for predominately female-functioning traits (Delph and Lively, 1992; Burd, 1994; Wilson et al., 1994). We call this the female function hypothesis for consistency. Although other gender-specific and sexual selection hypotheses exist to explain secondary traits (e.g., Ashman and Morgan, 2004), we limit our discussion to these two basic hypotheses. We provide numerous examples that support each hypothesis; first, we limit our discussion to plants that do not exhibit gender-biased trait expression, and later, we highlight the examples of gender-biased nectar production that are likely explained by one of the sexual selection hypotheses.

**Bateman’s Principles and the Male Function Hypothesis**

Sexual selection for exaggerated male phenotypes requires that the relationship between fecundity and mating success is stronger for males than females. Bateman (1948) provided the first experimental evidence of this relationship in his study of competition for mates in *Drosophila*. He showed that males exhibit a greater variance in both number of offspring and mating success, and he demonstrated a stronger correlation between these fitness components in males than females (reviewed in Arnold, 1994). These observations formed the basis of Bateman’s three principles of intra-sexual selection. Trivers (1972) expanded on Bateman’s principles and suggested that differential parental investment between males and females was the cause of mate limitation on male fecundity and resource limitation on female fecundity. Collectively, their work suggests that males should experience stronger sexual selection than females. When Bateman’s principles are specifically applied to hermaphroditic plants (Charnov,
The prediction arises that sexual selection acts on floral traits predominately through male function, because male fecundity is limited by pollinator visits and female fecundity is limited by the resources needed to set seeds (Arnold, 1994). This is the framework of the male-function hypothesis.

Experimental studies of a variety of secondary floral traits that lack gender-biased expression lend preliminary support to the male function hypothesis. For example, the amount of pollen removed or number of seeds sired is often limited by the number of pollinator visits to plants, whereas the number of seeds set is not (Queller, 1983; Bell, 1985; Queller, 1985; Sutherland, 1987). Further, at least one study has shown greater variance in mating success of male parents compared to female parents (Meagher, 1986). Thus, attractive floral traits of hermaphroditic flowers may serve a predominately male function in some species, as predicted by Bateman’s principles. Nectar production, in particular, benefits male function through its positive correlation with both the number of pollinator visits (Mitchell and Waser, 1992; Mitchell, 1993; Melendez-Ackerman and Campbell, 1998) and the amount of time spent per flower (Thomson, 1986; Jones and Reithel, 2001). In the hermaphroditic *Asclepias quadrifolia* and *Ipomopsis aggregata*, plants with increased nectar production receive more pollinator visits and export more pollen, but do not set more seeds (Pleasants and Chaplin, 1983; Mitchell, 1993). Plant species with unisexual flowers have also been cited in support of the male function hypothesis; many monoecious and dioecious species produce larger male flowers and/or have increased nectar production by males than females (Willson and Ågren, 1989; Delph and Lively, 1992; Eckert, 1999; but see Delph et al., 1996). In conclusion, studies of secondary traits in both hermaphroditic and unisexual species are often consistent with the male function hypothesis.
Beyond Bateman: Pollen Limitation and the Female Function Hypothesis

Bateman (1948) demonstrated that sexual selection was caused by mate limitation on male reproductive success. He did not predict stronger mate limitation on female reproductive success because females tend to be more dependent on resources for reproduction than access to mates. Nonetheless, resources do not always more strongly limit female function, as evidenced by the frequent occurrence of pollen limitation. Pollen limitation is the failure of a plant to achieve maximum female reproductive success because of insufficient pollen delivery – in terms of quantity and/or quality of pollen grains (Ashman et al., 2004). Burd (1994) reviewed studies of 258 plant species, and found evidence of pollen limitation in 62% of cases examined. A reanalysis of these and other data using phylogenetically independent contrasts (Larson and Barrett, 2000) and a recent review (Ashman et al., 2004) further support these findings.

In species with pollen limitation, secondary traits may often be more female-biased than male-biased in function. Nevertheless, there are only a few experimental demonstrations of sexual selection to increase female mating success (female function hypothesis), in the absence of gender-biased expression. Devlin and Ellstrand (1990) found variance in mating success to be greater for female rather than male function in *Raphanus sativa*. Selection through female fecundity was also stronger in *Asclepias syriaca*, based on data that showed differences between male and female selection gradients on floral traits (Morgan and Schoen, 1997). Strong correlations between female mating success and certain secondary traits were found in a congener, *A. exaltata* (Broyles and Wyatt, 1995). In this study, however, male mating success was also strongly dependent on the same traits. In summary, the ubiquity of pollen limitation suggests that selection may indeed act strongly through female function, but evidence for this hypothesis is currently quite limited.
The Sexual Selection Hypotheses in Plants with GBNP

Sexual selection in dichogamous plants with gender-biased nectar production is somewhat distinct from examples highlighted in the preceding paragraphs. In plants with gender-biased nectar production, the sexual selection hypotheses not only require a gender-differential relationship between mating success and fecundity, but they also suggest that pollinators are responding to reward differences and are visiting preferentially the more-rewarding floral gender. Pollinator preferences for certain flowers on a plant are known for some species on Table 2.1 and are highly likely for many others, as we will explain in the next section. Thus, in theory, the male-function and female-function hypotheses are respectively equipped to explain male- and female-biased nectar production. Nevertheless, only the male-function hypothesis is currently supported by empirical data.

Six species with male-biased nectar production have been considered within the context of sexual selection, and all are basically consistent with the major predictions of the male-function hypothesis. Of these species, *Impatiens capensis* (Balsaminaceae) provides a notable example. Flowers produce twice as much nectar and spend roughly twelve more hours in the male than female phase, which results in eight times as many visits to male-phase flowers (Bell et al., 1984). Although they did not explicitly test if male reproductive function was pollinator-limited in this plant species, Bell et al. (1984) stated that multiple pollinator visits were required to remove all of the pollen grains present on the anthers of male-phase flowers, whereas one or two visits to a female-phase flower were enough to deliver all the pollen necessary to fertilize all of its 3-5 ovules (see also Temeles and Pan, 2002). Thus, the nectar production pattern in *I. capensis* supports the male-function hypothesis. *Alstroemeria aurea* (Aizen and Basilio, 1998) and *Lobelia cardinalis* (Devlin and Stephenson, 1985), both with male-biased nectar production, also conform to predictions of this hypothesis. *A. aurea* required 3.3 visits to male-stage flowers
for every visit to female-stage flowers to maximize contributions of each sex, with 3.1 times as much nectar produced in the male stage (Aizen and Basilio, 1998). Studies of Agave mckelvyana, Bomarea acutifolia, Chrysothemis friedrichsthaliana and Echium vulgare provide some support for the male-function hypothesis as well. The remaining species with male-biased nectar production have yet to be studied in this context.

Despite the absence of empirical studies on most species, there are two lines of indirect evidence that sexual selection is a probable explanation for at least some species with gender-biased nectar production. For example, gender-biased expression of nectar is often accompanied by a similarly biased expression of other floral characteristics. Flowers of Impatiens capensis show increased longevity of the more-rewarding male phase (Bell et al., 1984). Petals of other species begin to wilt notably during the less-rewarding female phase (McDade, 1986; J. E. Carlson, unpublished data), or they change color as they pass into the less-rewarding female-phase (Klinkhamer and de Jong, 1990). The concurrent biasing of multiple traits provides some indirect support to the sexual selection hypotheses.

An additional line of indirect evidence relies on specific behaviors of the pollinators. If sexual selection is currently maintaining gender-biased nectar production, pollinators must be able to distinguish male- and female-phase flowers. They also must visit preferentially flowers of the more-rewarding phase. Based on current information on the species of our lists, pollinators appear to prefer flowers in the more-rewarding phase for seven species with male-biased nectar production (five of these are without nectar gradients) and eight with female-biased nectar production (two of these are without nectar gradients; Table 2.1). For species that have discriminating pollinators, increased rewards can result in increased mating success, which would allow nectar to be a sexually selected trait. When pollinators do not discriminate or intrasexual competition seems unlikely, the evolution of gender-biased nectar production may
more closely correspond with an alternative possibility, based on natural selection against inbreeding.

**Inbreeding Avoidance Hypotheses**

Floral traits that reduce maladaptive inbreeding are favored by natural, rather than sexual, selection. This is because such traits increase offspring quality per mate, but generally not mating success. Inbreeding occurs whenever pollen fertilizes the ovules of its own flower (autogamy) or ovules of other flowers on the same plant (geitonogamy). In either case, both male and female components of fitness may be drastically reduced by the production of fewer or inferior offspring by inbred flowers (Charlesworth and Charlesworth, 1987). Inbreeding further reduces male reproductive success due to pollen discounting, which is the loss of pollen that could have sired outcrossed seeds (de Jong et al., 1993; Fishman, 2000). Both Rathcke (1992) and Harder et al. (2001) suggest that reproductive losses to geitonogamy are more severe for male than female fitness, providing an opportunity for gender-specific selection in the absence of sexual selection. Mating among flowers on the same plant is thought to be a very common mode of self fertilization (Lloyd, 1992), making it an important, although frequently ignored force in floral evolution (de Jong et al., 1993).

According to the inbreeding avoidance hypotheses, gender-biased nectar production is an adaptation to decrease geitonogamous inbreeding through its effects on pollinator behavior. Maximal pollen export (i.e., minimal inbreeding) is predicted when many individual pollinators each visit only a small fraction of the available flowers on a plant (Iwasa et al., 1995; Ohashi and Yahara, 2001), and when each pollinator visits flowers in a particular order, if flowers are dichogamous. Pyke (1978a), Feinsinger (1978) and Rathcke (1992) proposed that these behaviors could be triggered by within-plant variation in nectar rewards, as occurs in plants with
gender-biased nectar production. Given that pollinators respond as expected, gender-biased rewards should reduce geitonogamous self-pollination.

The predictions of the inbreeding avoidance hypotheses for gender-biased nectar production are as follows: there are sufficient costs of inbreeding through within-plant pollen movement, and there is a particular response from the pollinators. Of these predictions, the effects of inbreeding provide the primary dichotomy between the inbreeding avoidance and the sexual selection hypotheses. We nevertheless focus the following discussion on the second requirement of the inbreeding avoidance hypotheses, that of pollinator foraging behavior, and specifically, the behavior of pollinators foraging for nectar rather than pollen. A distinct set of pollinator foraging behaviors provides the means by which gender-biased nectar production may be favored.

**Predictions Derived from Foraging Theory**

Foraging theory predicts that pollinators use information and experience to make decisions that optimize energy intake and maintain a positive energy balance, i.e., optimal foraging strategies (Best and Bierzychudek, 1982; Hodges, 1985). Two strategies are particularly relevant for pollinators foraging on plants with variable rewards: absolute or probabilistic threshold departure rules (based on the marginal value theorem; Charnov, 1976; Pyke et al., 1977; Stephens and Krebs, 1986; Dreisig, 1989; Pappers et al., 1999) and risk-sensitive behavior, perhaps better described as avoidance of unpredictable rewards (Stephens and Krebs, 1986; Kacelnik and Bateson, 1996). Each of the above strategies corresponds to one of our two inbreeding avoidance hypotheses. The first, called the declining rewards hypothesis, predicts that a pollinator often visits flowers in order of declining reward quality, and it stops inspecting flowers on a plant if it encounters flowers below a threshold value of nectar. The second, the unpredictable rewards hypothesis, states that a pollinator does not visit flowers in any
particular order because rewards are unpredictable, and it departs a plant once it detects a
variable nectar supply among flowers on that plant. Using either strategy, pollinator responses to
variability may reduce geitonogamy, but the actual cues used differ depending on the strategy.
In the following two sub-sections, we describe these two inbreeding avoidance hypotheses, and
we evaluate how well they apply to gender-biased nectar production.

The Declining Rewards Hypothesis

A pollinator may use rules to visit and depart from a plant with or without previous
knowledge of rewards. Nevertheless, optimal foraging theory assumes that a nectar-feeding
animal knows which plants and flowers within its diet are most rewarding and exploits these
options accordingly (Stephens and Krebs, 1986). The assumption of a well-informed pollinator
is supported by observational and experimental data (Gass and Sutherland, 1985; Hurly, 1996;
Garrison and Gass, 1999; Healy and Hurly, 2001). We will therefore begin our discussion of the
declining rewards hypothesis under the assumption that pollinators have some preconception of
reward quality before visiting the flower.

The declining rewards hypothesis predicts that pollinators foraging on variable
inflorescences behave in two particular ways to maximize the rate of energy gain. First,
pollinators will direct movements within inflorescences from more to less-rewarding flowers.
This prediction is well-supported by foraging studies, particularly those using bees foraging on
racemes with nectar gradients (Pyke, 1978a, 1978b; Best and Bierzychudek, 1982; Galen and
Plowright, 1985b; Hurly, 1996; Evans, 1996). Second, pollinators should depart before visiting
relatively poor options on the inflorescences. This behavior, known as complete or partial
preferences, is commonly observed across pollinator taxa (Best and Bierzychudek, 1982; Bell et
al., 1984; Devlin and Stephenson, 1985; Galen and Plowright, 1985a, 1985b; Klinkhamer and de
Jong, 1990; Kadmon et al., 1991; Cresswell and Robertson, 1994; Gonzales et al., 1995; Aizen
and Basilio, 1998). Thus, empirical evidence shows that pollinators often – but not always – leave the inflorescence before visiting less-rewarding flowers.

The above rate-maximizing behaviors are likely to reduce inbreeding only when gender-biased nectar rewards are distributed appropriately among flowers. That is, if female-phase flowers are more rewarding, then pollinators should move from female to male within plants and visit fewer males overall, causing little geitonogamy. If male-phase flowers are more rewarding, however, rates of geitonogamy may be relatively high. If flowers are equally rewarding, an intermediate degree of inbreeding is expected. Thus, female-biased rewards may best reduce inbreeding when rewards are predictable, relative to constant or male-biased rewards.

When pollinators cannot or do not discriminate, they may still use threshold departure rules, which should reduce geitonogamy if rewards are female-biased. Departure from a plant with an unknown reward distribution still follows a visit to a relatively unrewarding flower (Hodges, 1985; Dreisig, 1989) or short series of flowers (Cresswell, 1990; Pappers et al., 1999), but the pollinator does not target the most rewarding flowers at any point during the bout. Pollinators may chose not to discriminate if the rewarding gender is in the majority for the population (Schemske et al., 1996; Castillo et al., 2002) or if discrimination is useless (e.g., Pappers et al., 1999) or costly (Bell, 1986; Gilbert et al., 1991; Smithson and Gigord, 2003). In any case, the declining rewards hypothesis is still a viable explanation of gender-biased rewards.

**The Unpredictable Rewards Hypothesis**

The unpredictable rewards hypothesis states that pollinators foraging on variable inflorescences are risk-aversive, which should result in reduced plant inbreeding. Risk-aversive behavior is a foraging strategy in which a pollinator attempts to maximize nectar intake by avoiding variable or ‘risky’ alternatives while foraging (Real, 1981; Kacelnik and Bateson, 1996). This body of theory assumes that the reward value of individual flowers on variable
plants (or patches of plants) cannot be predicted by pollinators, which is a particularly unattractive possibility for pollinators seeking a positive energy budget (Caraco et al., 1980; Kacelnik and Bateson, 1996). For these foragers, the relationship between energy intake rate and fitness gains is often concave-down, which means that the energetic value of a food item decreases as satiation is approached (Stephens and Krebs, 1986; Kacelnik and Bateson, 1996). Given this relationship, the best foraging strategy is to avoid variable plants altogether (i.e., risk-aversion), because the fitness benefit of choosing a more rewarding flower on a variable plant is outweighed by the cost of choosing a less rewarding flower (known as Jensen’s inequality; Stephens and Krebs, 1986).

There is strong evidence that both vertebrate and invertebrate pollinators employ risk-aversive foraging strategies (reviewed in Kacelnik and Bateson, 1996). Most experiments demonstrate that pollinators avoid variable inflorescences, which are distinguishable based on color cues (Waser and McRobert, 1998; Hurly and Oseen, 1999). In other words, pollinators are often found to be risk-averse between inflorescences. Nevertheless, Biernaskie et al. (2002) showed that cryptic variability also can elicit risk-aversive behavior within inflorescences, such that departure is earlier from variable inflorescences. For risk-aversive behavior to pertain to within-inflorescence movements, however, we stress that pollinators must detect variability per se and depart from inflorescences following rewarding and unrewarding individual flowers with equal frequencies. If departure from variable inflorescences more often follows an unrewarding visit, then these behaviors are more congruent with threshold departure rules than risk-aversive behaviors.

Under the unpredictable rewards hypothesis, risk-aversive pollinators decrease inbreeding in plants with variable rewards because they visit only a fraction of available flowers before departure. Here, variable rewards may be achieved by many nectar production patterns,
including both male-biased and female-biased nectar production, as long as the reward status of flowers remains unpredictable to pollinators. If variable rewards are to be favored by selection, pollinator responses to variance must decrease inbreeding to the extent that plant reproductive success is increased relative to constant rewards. Indirect evidence that risk-aversive behavior reduces plant inbreeding is limited to a single study using artificial inflorescences. Biernaskie et al. (2002) found that both bees and hummingbirds visited fewer flowers on artificial inflorescences with variable as opposed to constant rewards. They concluded that similar responses to variability would reduce geitonogamy on real plants, but this has yet to be demonstrated.

The Inbreeding Avoidance Hypotheses in Plants with GBNP

The inbreeding avoidance hypotheses are equipped to explain both male- and female-biased nectar production, but these hypotheses are currently supported by only female-biased examples. Female-biased nectar production has been hypothesized to reduce inbreeding via the declining rewards hypothesis in six different bee-pollinated species. Four herbs with female-biased nectar production and vertical inflorescences (Aconitum columbianum, Delphinium barbeyi, D. nelsonii, Epilobium angustifolium) provided the data used to formulate this hypothesis (Pyke, 1978b). Pyke predicted that the upward direction of foraging and quick departure following a male-phase visit would cause little geitonogamy and was an adaptive plant trait. Waddington (1981) came to similar conclusions using D. virescence. The best evidence for this hypothesis comes from a study by Best and Bierzychudek (1982) using Digitalis purpurea. Bee movements were shown to match almost perfectly the predictions of the declining rewards hypothesis: pollinators visited first female-phase flowers and moved on to visit relatively fewer male-phase flowers. They concluded that “the existing pattern of nectar
presentation is evidence that natural selection has favored careful regulation of the amount of
reward in individual Digitalis flowers” (Best and Bierzychudek, 1982, pg. 78).

The inbreeding avoidance hypotheses are unlikely to apply to some species with gender-biased nectar production. For example, Agave, Alstroemeria, Bomarea, and Pentagonia are protandrous at the whole-inflorescence level, such that all flowers are male, then female, with no overlap. Thus, geitonogamy is probably rare or impossible in these species, such that selection on nectar production patterns would not be associated with inbreeding avoidance. Male-biased nectar production in general cannot be well-explained by the declining rewards hypothesis, although it may be explained by the unpredictable rewards hypothesis. The unpredictable rewards hypothesis must be disregarded, however, if pollinators are able to discriminate and preferentially visit the more-rewarding flowers.

Variable rewards resulting from gender-biased nectar production theoretically need not be associated with cues nor induce departure following a relatively unrewarding visit. Nevertheless, the only existing evidence for inbreeding avoidance comes from plants that do both. This suggests that the declining rewards hypothesis may be more relevant than the unpredictable rewards hypotheses to plants with gender-biased nectar production. If true, male-biased nectar production in most species is probably not an adaptation to reduce inbreeding depression. Based on current evidence, inbreeding avoidance hypotheses should most directly apply to species with female-biased nectar production, upward decreasing nectar gradients, and most importantly, a high cost of inbreeding via geitonogamy.

Plants with female-biased nectar production do appear to receive fewer male-to-female visits within the plant, but thus far, reduced inbreeding is only inferred from directionality. Fortunately, evidence that nectar (or lack thereof) can directly decrease geitonogamy does, however, exist in another plant group: the nectarless orchids. The production of rewardless
flowers, like the production of variable rewards, is considered an adaptation to reduce consecutive within-plant visits, to ultimately reduce inbreeding (Dressler, 1981). This hypothesis has been explicitly tested in a few nectarless orchids (e.g., *Orchis mascula*, Johnson and Nilsson, 1999; *Disa pulchra*, Johnson, 2000), and is currently best supported by a recent study of *Anacamptis morio* (Johnson et al., 2004). *A. morio* inflorescences with rewardless flowers received fewer within-plant visits and thereby experienced less geitonogamy, relative to inflorescences with artificially induced rewards in flowers. Thus, natural selection can favor alternative nectar production patterns to result in reduced inbreeding, but evidence for such an effect in plants with gender-biased nectar production is currently lacking.

**OTHER FACTORS INFLUENCING THE EVOLUTION OF GBNP**

Sexual selection and natural selection against inbreeding are the best supported explanations for gender-biased nectar production, and they consequently form the two central themes of our review. Nevertheless, three additional factors may also be highly relevant to the evolution of gender-biased nectar production on a case-by-case basis. These factors are resource allocation trade-offs, the effects of floral enemies, and pleiotropy. In the following subsections, we briefly describe each factor and its expected significance to the evolution of gender-biased nectar production. We have found no studies that explicitly link resource-allocation tradeoffs, floral-enemy effects or pleiotropy to the evolution of gender-biased nectar production, and thus, empirical support for these possibilities is quite limited.

**Resource-Allocation Tradeoffs**

Costs associated with floral nectar production may impede directional selection on nectar production rates. Plant energetic resources may be insufficient to simultaneously increase nectar production, develop all fertilized ovules and carry out all other essential biological functions. As a result, additional resources allocated towards nectar production must thereby be siphoned from
other resource sinks, potentially negating the reproductive benefits of increased pollinator attraction. Evidence for a resource-allocation tradeoff involving nectar has been found in *Asclepias syriaca* (Southwick, 1984) and *Blandfordia nobilis* (Pyke, 1991), but not in *Echium vulgare* (Klinkhamer and van der Veen-van Wijk, 1999; Leiss et al., 2004).

Haig and Westoby (1988) examined the resource-allocation tradeoff between reproduction and attraction from an evolutionary perspective. They predicted that plants should be selected to distribute resources such that reproductive output is limited simultaneously by both pollinators and resources. Resource allocation towards nectar production is therefore maintained at an optimal level; one that balances sufficient pollinator attraction with maximal ovule development. Thus, nectar production should stay constant, unless selection for augmentation is coupled with new, more efficient resource allocation strategies that increase resource provisioning to both plant functions.

Resource-allocation tradeoffs may limit the production of additional nectar during one floral phase, which may impede selection for gender-biased nectar production in some plants. Nevertheless, a net increase in nectar production is not a necessary requirement of gender-biased nectar production. For example, increased allocation to nectar production during one phase may be countered by decreased allocation to nectar production during the other, resulting in no net change in nectar production rates. Because the ancestral nectar production patterns of plants currently with gender-biased nectar production are unknown, the role of resource allocation tradeoffs in their evolution is difficult to reconstruct. We conclude that resource-allocation tradeoffs alone are unlikely to promote or impede selection for gender-biased nectar production, yet they likely interact with other selection pressures to influence nectar production rates. Their combined result may be gender-biased nectar production with no net change in lifetime nectar production of flowers, relative to original conditions.
Effects of Floral Enemies

Floral enemies, such as nectar robbers, pathogens and herbivores, have occasionally been implicated in the evolution and maintenance of floral traits (Linhart, 1991; Brody, 1997; Strauss, 1997; Shykoff et al., 1997; Galen, 1999). Here, we consider the possibility that nectar robbers and pathogens, in addition to pollinators, have been important selection agents favoring gender-biased nectar production.

Nectar robbers and pathogens may alter the outcome of selection on floral traits by rendering disadvantageous the traits that are initially most attractive to pollinators. Nectar robbers – birds or arthropods that steal nectar without pollinating the plant – may visit preferentially flowers with higher nectar rewards (Galen, 1999). Consequently, the most attractive flowers are those most likely to experience any reproductive costs of robbery, either due to nectar robber damage to developing seeds (Traveset et al., 1998; Galen, 1999) or, more commonly, through pollinator avoidance of robbed flowers (Roubik, 1982; Irwin and Brody, 1998; Irwin et al., 2001). Floral pathogens that use pollinators as vectors may affect floral trait evolution in a similar way (Shykoff and Bucheli, 1995; Shykoff et al., 1997). An increased risk of infection and sterilization for attractive plants is likely to counter possible benefits of increased pollinator visits when floral pathogens are involved.

If nectar robbery or floral pathogens are particularly damaging to the plant (through either male or female function), it may be beneficial to make flowers less attractive. This may be achieved by providing a smaller or less appealing nectar reward (Willson and Ågren, 1989; Adler, 2000). In species with gender-biased nectar production, a smaller reward may equate to reduced nectar production in one of the two sexual phases. The sexual phase whose fecundity is decreased more by robbery (or fungal infection) is predicted to have relatively smaller rewards, based on expectations that selection is gender-specific (Willson and Ågren, 1989; Irwin and
Brody, 2000). Even in the absence of gender-specific selection, nectar may be biased away from the first phase (regardless of gender) if robbery is prolonged or signs of robbery are carried over to the next phase. In summary, gender-biased nectar production may be selected for by enemies if reduced nectar production during one sexual phase decreases damaging visits to flowers of that phase and thereby increases total plant fitness.

Selection imposed by floral enemies may explain gender-biased nectar production, but only in a limited spectrum of plants. First, plants must suffer a high cost of nectar robbery or disease to male function, female function or both. Costs of robbery have been demonstrated for only a few plant species, and their relative importance to total reproductive success is a matter of debate (Maloof and Inouye, 2000; Irwin et al., 2001; Irwin and Maloof, 2002). The costs of a sterilizing fungal disease are less contentious (Shykoff et al., 1997). Second, the effects of floral enemies on a plant must decrease with decreasing nectar rewards during the male or female phase, which in turn must increase total reproductive output relative to plants with unbiased rewards. In *Impatiens capensis*, female-phase flowers produce less nectar than male-phase flowers, and these flowers also experience decreased nectar robbery (Temeles and Pan, 2002). For *I. capensis*, however, male-biased nectar production is probably not a result of selection for increased female output through decreased nectar robbery during the female phase. This is because the gender-specific costs of robbery to *I. capensis* are likely countered and overwhelmed by gender-specific selection for increased pollen removal by pollinators (Bell et al., 1984; Temeles and Pan, 2002). In *I. capensis* and similar examples, selection by enemies is unlikely to override selection by pollinators. This may account for the paucity of evidence in favor of an enemy-associated effect on nectar production. Nonetheless, floral enemies may conceivably contribute to the overall strength of selection on nectar production patterns.
Pleiotropic Effects

Floral traits may evolve by selection acting on other, unrelated traits. These pleiotropic relations may explain gender-biased nectar production, particularly when this nectar production pattern does not appear to benefit the plant (i.e., unbiased rewards confer higher total fitness). Although genetic correlations due to pleiotropic effects are difficult to identify, phenotypic correlations between nectar production and other plant traits have been measured in many cases. Nectar volume is phenotypically correlated with many plant and flower traits, including corolla dimensions (positive correlation; Ashman and Stanton, 1991; Galen, 1999), root weight (positive correlation; Pleasants and Chaplin, 1983), and umbel size (negative correlation; Pleasants and Chaplin, 1983). Nectar volume is genetically correlated with a more limited subset of traits, including nectar sugar concentration (Klinkhamer and van der Veen-van Wijk, 1999) and flowering date (Mitchell and Shaw, 1993). Very few data are available on genetic correlations among floral traits, but those that exist suggest selection on correlated traits has the potential to influence floral evolution (Mitchell, 2004).

Phenotypic correlations may occur between the same trait in male and female individuals, in individuals in different environments (Roff, 1997), or in the male and female phase of the same flower. In species with gender-biased nectar production, nectar production rate and maturation of male and female parts necessarily vary together. In the protandrous plants *Streptanthus carinatus* and *S. culteri*, flowers do not open completely until they enter the female phase (Rollins, 1963). In this case, maturation of the nectaries likely matches that of the gynoecia, which results in female-biased nectar production. If the above phenotypic correlation between nectary and gynoecial development reflects a true pleiotropism, selection for traits causing protandry may thereby be the driving force behind female-biased nectar production in this species, rather than selection on the nectar production pattern itself. Secondary traits with
gender-biased expression, such as color, corolla morphology and pollen rewards may also be pleiotropically linked to nectar production during a floral phase, and they are often subject to strong selection independent of nectar rewards (Waser and Price, 1981; Campbell et al., 1996; Melendez-Ackerman et al., 1997; Cresswell, 1999). When the true target of selection is developmentally linked through pleiotropy to nectar, gender-biased nectar production may arise and be maintained as a non-adaptive trait.

**FUTURE DIRECTIONS**

In this paper, we highlight plants with gender-biased nectar production for their potential to advance various lines of research within floral biology and evolution. To date, few of these species have been subject to intensive study, and for many, none of the hypotheses recognized by this paper have been tested. Below, we outline a series of experiments and predictions that, when performed together, should indicate the hypothesis (or hypotheses) that best explains gender-biased nectar production in a particular species. We assume here that gender-biased nectar production is a heritable trait, but suggest that this assumption be explicitly tested for each species studied. The experiments we propose pertain to two categories: first, the study of plant reproductive biology and second, the study of pollinator behavior. The former category may be further divided into tests for intrasexual selection and for fitness costs due to inbreeding.

**Tests of Hypotheses for GBNP Using Plants**

**Detecting Intrasexual Selection in Plants**

To assess intrasexual competition for pollinators, the relationships between mating success and male versus female reproductive output must be measured, and the strengths of these relationships may then be compared to nectar production rates during each sexual phase. In addition, it must be shown that nectar production and pollinator visits are directly related. The experimental manipulation of nectar may be used in direct tests of sexual selection hypotheses.
If the augmentation of nectar in a floral phase increases pollinator visits to that phase, and increased visits differentially affect male and female reproductive output (i.e., there is an interaction between sexual phase and nectar addition), then there may be intrasexual competition for pollinators. If nectar production is male-biased and male reproductive output is more strongly limited by nectar production (and pollinator visits) than is female reproductive output, then the male-function hypothesis is supported. If nectar production is female-biased and female reproductive output is the more strongly nectar-limited, then the female-function hypothesis is supported.

Assessing the Costs of Inbreeding in Plants

To explore the inbreeding avoidance hypotheses from the plant perspective, the potential for inbreeding via geitonogamy and inbreeding depression must be measured. Because inbreeding may have gender-biased effects, its consequences must be measured separately for male and female reproductive output, in terms of both pollen discounting and inbreeding depression. If a plant population is self-compatible, has male- and female-phase flowers open simultaneously, and produces fewer offspring or offspring of poorer quality when inbred as opposed to outcrossed, this is preliminary support for both of the inbreeding avoidance hypotheses. Decreased inbreeding, as cause by gender-biased nectar production, must also be linked with increased plant reproductive success. Nectar manipulations again may serve to tease apart the relationships among gender-biased rewards, reproductive losses to inbreeding and reproductive gains through outcrossing. In this case, however, pollen tracking or paternity analysis is needed to determine the number of inbred versus outcrossed offspring produced by plants receiving different nectar treatments.
Tests of Hypotheses for GBNP Using Pollinators

Reproduction in animal-pollinated plants cannot be fully understood without consideration of the pollinator. Similarly, most of our hypotheses cannot be fully tested without some measurement of pollinator responses. We therefore suggest that researchers quantitatively assess pollinator foraging behavior on plants with gender-biased rewards, to test specific assumptions of the sexual selection and inbreeding avoidance hypotheses. Below, we explain how the present-day responses of pollinators should reflect the relative likelihood of the different evolutionary hypotheses for gender-biased nectar production. If the selection environment has changed drastically, these observations should at least reflect the role of pollinators in the maintenance of the trait today.

Distinguishing Between Alternatives Using Pollinator Foraging Behavior

Both the sexual selection and inbreeding avoidance hypotheses are based on the assumption that a pollinator is unlikely to visit all flowers on a multi-flowered plant (both male and female flowers present) during every visit to the plant. If true, then knowledge of partial preferences and directionality should facilitate discrimination among most evolutionary hypotheses. For example, under the sexual selection hypotheses, pollinators must exhibit partial preferences for the more-rewarding flower, which in this case implies that they are able to distinguish male- and female-phase flowers (based on spatial location or visual cues). Under the declining rewards hypothesis, an informed and discriminating pollinator is not an essential requirement, although it is anticipated, based on optimal foraging theory. Under the unpredictable rewards hypothesis, in contrast, a pollinator cannot be informed or discriminating, and all flower types may be visited at roughly equal frequencies.

Sexual selection and inbreeding avoidance hypotheses also differ in the required directionality of within-plant movements made by pollinators. Under the sexual selection
hypotheses and the unpredictable rewards hypothesis, there is no required order of visits, such that male-to-female moves may be as frequent as female-to-male moves, regardless of gender-biased direction. Under the declining rewards hypothesis, however, pollinators are required to move from higher rewards to lower rewards within the plant. Nevertheless, these criteria are unlikely to allow investigators to completely discriminate between sexual selection and inbreeding avoidance hypotheses. This is because both directionality and frequency may be similar under either set of hypotheses. Pollinators often (but not always) visit more-rewarding flowers first, and depart before visiting less-rewarding flowers (required by declining rewards and permitted by sexual selection), which results in a higher frequency of visits to more-rewarding flowers (required by both sexual selection and declining rewards). These comparisons, in combination with results from the plant reproductive biology experiments, should, nevertheless, allow elimination of hypotheses that are unlikely to have contributed to gender-biased nectar production in individual species.

CONCLUDING REMARKS

In many influential articles and reviews, Willson (1979, 1990, 1994) has argued that sexual selection is an important yet generally underappreciated force in the evolution of floral traits. We concur and endorse sexual selection as a potential explanation for gender-biased nectar production in many hermaphroditic plants. Nonetheless, we find sexual selection hypotheses insufficient to completely explain all examples of gender-biased nectar production, and we contend that hypotheses based on selection against inbreeding are important alternative or complementary explanations for many species. We cite available data on plants with female-biased nectar production and nectar gradients along vertical racemes to support these claims. We also stress that both sets of hypotheses are not mutually exclusive, and more than one hypothesis may apply to a single species. Finally, we propose that other factors, such as pleiotropy or floral
enemy effects, may also be of importance in the evolution and maintenance of gender-biased nectar production, particularly if the primary hypotheses are not supported.

We acknowledge that no single test discriminates between the different hypotheses, and more than one hypothesis may apply simultaneously. Nonetheless, we have proposed sets of tests that together address in detail the leading evolutionary hypotheses for gender-biased nectar production in hermaphroditic plants. We stress that all possibilities warrant consideration in future studies and that the few well-established examples should perhaps be reexamined to definitively rule out alternative hypotheses. Completing the causal links among nectar production patterns, pollinator behavior and male and female components of plant fitness promises to be a complex task, but it is also an important and timely one. In addition to providing unique insights into gender-biased expression and function of floral traits, such research has the potential to unify diverse fields. Multiple disciplines, including pollinator cognitive biology, plant reproductive biology and floral evolution, must all be brought to bear if we are to fully understand the underlying causes of gender-biased nectar production as a functional, evolved trait.

**LITERATURE CITED**


Chapter 3
Male-Biased Nectar Production in a Protandrous Herb Matches Predictions of Sexual Selection Theory in Plants

INTRODUCTION

Floral nectar positively affects pollination success and fitness of many flowering plants. Pollinator visits typically become longer and more frequent as nectar production increases (Hodges, 1985; Mitchell and Waser, 1992; Burd, 1995; Hodges, 1995; Melendez-Ackerman et al., 1997), which often results in a positive correlation between nectar and fecundity estimates of individual flowers or plants (Zimmerman, 1983; Thomson, 1986; Mitchell, 1993; Jones and Reithel, 2001; for exceptions, see Hodges, 1995; Salguero-Faria and Ackerman, 1999). In plants with hermaphroditic flowers, the amount of nectar influences important components of male and female reproductive success (Mitchell and Waser, 1992; Jones and Riethel, 2001), yet fitness consequences may be sexually asymmetrical within plants. Specifically, male fitness is often (but not always) more strongly associated with nectar production rates/schedules than is female fitness (Pleasants and Chaplin, 1983; Mitchell, 1993; Aizen and Basilio, 1998).

A male-biased benefit of nectar is predicted from sexual selection theory (reviewed in Stephenson and Bertin, 1983; Bell, 1985; Arnold, 1994; Willson, 1994; Skogsmyr and Lankinen, 2002; Carlson and Harms, 2006), which assumes that male fitness is most strongly limited by access to mates, whereas female fitness is most strongly limited by resources (Darwin, 1871; Bateman, 1948; Charnov, 1979). Attractive structures may therefore be more beneficial to males (or male function, in hermaphrodites) because they increase mating success through pollinator visits, but usually lack photosynthetic tissue to provision fruits and seeds. Furthermore,
reproductive assurance through autogamy allows some hermaphroditic plants to sire and set seed in the absence of visits (Jarne and Charlesworth, 1993), but even full seed set through selfing is unlikely to require all of a flower’s pollen. Although there is evidence that female fitness is often strongly limited by outcrossing opportunities (see Burd, 1994; Wilson et al., 1994; Ashman and Morgan, 2004), fundamental differences in gamete size and reproductive investment suggest that males will be more strongly limited by access to mates than females in most plant and animal species (Bateman, 1948; Trivers, 1972; Queller, 1997). Direct tests of this relationship are nevertheless necessary, given that pollination limitation of female fecundity is widespread (e.g., Ashman et al., 2004).

Sexual selection for attractive floral traits is mediated by discriminating pollinators, and it is caused by stronger intramale competition for their visits. Traits that promote repeated, high-quality pollinator visits are selectively favored through male but not necessarily female fitness (Lloyd and Yates, 1982; Bell, 1985; Devlin and Stephenson, 1985; Willson, 1994; Aizen and Basilio, 1998). A likely result of these processes is a stronger association between secondary floral traits and male fecundity. Male-biased reproductive benefits are known for floral traits such as nectar quantity and quality, flower color, corolla shape, and inflorescence size (Bell, 1985; Stanton et al., 1986; Campbell, 1989; Galen and Stanton, 1989; Melendez-Ackerman and Campbell, 1998; Burd and Callahan, 2000), but convincing evidence that these traits are sexually selected is as yet limited. Because attractive traits of hermaphroditic flowers coincide with and potentially benefit both genders, the outcome of sexual selection is not strikingly sexually dimorphic, as it is in dioecious and monoecious plants (e.g., Willson and Ågren, 1989; Delph et al., 1996; Eckhart, 1999; Ashworth and Galetto, 2002).

In dichogamous plants, sexual selection has the potential to cause more sexual dimorphism than in other plants with hermaphroditic flowers. The separation of sexual phases in
time allows nectar to become temporally partitioned to match the disparate visit requirements of male- and female-phase flowers. In a recent review, Carlson and Harms (2006) found that 21 dichogamous plant species produce more nectar during the male phase. Evolutionary hypotheses have been considered in six of these species (Bell et al., 1984; Devlin and Stephenson, 1985; Sutherland, 1987; Klinkhamer and de Jong, 1990; Evans, 1996; Aizen and Basilio, 1998; see also Rathcke, 1992), yet explicit a priori tests of hypotheses for nectar asymmetry are virtually absent from the literature (Carlson and Harms, 2006; but see Biernaskie et al., 2002).

In this study, I documented male-biased nectar production and pollination in the dichogamous herb *Chrysothemis friedrichsthaliana* (Hanst.) H.E. Moore (Gesneriaceae) and compared observed patterns with four basic predictions of sexual selection theory in dichogamous plants: (1) nectar production should be persistently biased towards the male phase; (2) mating opportunities, achieved through pollinator visits, should be sufficiently limiting during all or part of the flowering season such that plants must compete for visits to maximize paternal fitness; (3) pollinators should respond to the nectar bias and visit male- more often than female-phase flowers; and (4) male fecundity should be more pollinator limited than is female fecundity, and autonomous self-pollination may further reduce visit requirements for maximal seed set.

**MATERIALS AND METHODS**

**Species and Study Site**

*Chrysothemis friedrichsthaliana* is a perennial herb endemic to the lowlands of Central America and northern South America (Skog, 1978). The plant is terrestrial or infrequently epiphytic, and it grows along the edges of forests, clearings, and streams, where it may form dense patches of hundreds of reproductive individuals. Flowering occurs throughout the rainy
season in Costa Rica (May to November) and peaks between July and August at a rate of one flower per day (Skutch, 1992; Chapter 1).

The flowers of *C. friedrichsthaliana* are protandrous and last 2 d (Skutch, 1992). The male phase begins shortly before sunrise on the first day, when all four anthers dehisce and the flower opens. Anthers remain at the mouth of the corolla until nightfall of the first day, at which time they begin to curl back into the tube of the corolla. The female phase begins, in effect, on the morning of the second day, when the stigma is receptive and has replaced the anthers at the mouth of the corolla. At the end of the female phase, the corolla detaches and falls to the ground (Skutch, 1992). Flowers are self-compatible, and self-fertilization may occur when pollen-bearing anthers curl back at the end of the male phase. Each phase lasts 12 daylight hours on average, although the duration of the female phase is variable and shortened by pollinator visits, but not by self-fertilization (Chapter 1).

The corolla of *C. friedrichsthaliana* is 2.5 cm long, orange with red nectar guides, tubular, narrow, and subtended by an enlarged, light-green, cup-like calyx. Nectar is produced by a bilobed nectary located at the base of the corolla (Skog, 1978; Skutch, 1992). The only important pollinator of *C. friedrichsthaliana* in the study population is the stripe-throated hermit, *Phaethornis striigularis* (Chapter 1). *Phaethornis striigularis* is a small, nonterritorial hummingbird of neotropical lowlands that forages on the nectar of a wide range of insect- and hummingbird-pollinated flowers (Skutch, 1951; Hinkelmann and Schuchmann, 1997). At least 17 different *P. striigularis* individuals made use of the plants in the study population during each full season of study (Chapter 1).

The study site is in the Golfo Dulce Forestal Reserve (8°42′ N, 83°31′ W) on Osa Peninsula, Puntarenas Province, Costa Rica. The reserve is lowland tropical wet forest (Holdridge, 1967) and receives 5.5 m rainfall per year (Kappelle et al., 2003). Experiments were
carried out on large (>1000 individuals), dense, nearly continuous *C. friedrichsthaliana* patches surrounding Fundación Neotrópica’s Centro Tropical, in Agua Buena de Rincón.

### Nectar Production Rates

To assess the strength and temporal pattern of the nectar bias in *C. friedrichsthaliana*, I measured nectar production in two ways. In both, I assessed nectar production in bagged flowers rather than flowers open to visits (e.g., standing crop), because increased visits to flowers of one phase could mask true production biases. First, I compared nectar accumulation in male and female-phase flowers bagged throughout their lifetimes. Second, I compared nectar replenishment rates of male- and female-phase flowers depleted of nectar and bagged 2 h before measurement. In a subset of these flowers, I applied pollination treatments prior to measurement, to determine whether outcrossed pollen affected nectar replenishment.

### Nectar Accumulation Rates

I measured the nectar accumulated in unvisited flowers of different ages on 16 plants. From mid-July to mid-August 2004, all buds were bagged the afternoon before opening with fine, green polyester netting (8 × 12 mesh count per cm²) and left undisturbed until measurement. Randomly assigned measurement times were: the morning (0 h after sunrise, i.e. 0530 hours, local time), mid-day (6 h after sunrise), or evening (12 h after sunrise) of the male phase, or morning (24 h after sunrise of the morning of the male phase), mid-day (30 h), or evening (36 h) of the female phase. Each measurement time was represented by at least three flowers per plant (*N* ≥ 48 per measurement time).

At measurement, flowers were removed from plants, and nectar volume and percentage of sugar, or sucrose equivalents, were measured within a half hour. Nectar was extracted and measured with 10-µL calibrated microcapillary tubes and calipers, and sugar concentration was measured with a portable sugar/brix refractometer with automatic temperature compensation and
a range of 0–32% (Sper Scientific, Scottsdale, Arizona, USA). Sugar concentration was converted to grams of sugar per liter of nectar (Kearns and Inouye, 1993) and multiplied by nectar volume to obtain mass of sucrose equivalents, or mass of sugar. I estimated nectar production rates in unvisited male- and female-phase flowers over preceding night, day, morning, and afternoon intervals by subtracting the mean amount of sugar in flowers at the end of each interval from that at the beginning of each interval.

I performed analysis of variance (ANOVA) on daytime and nighttime nectar production in unvisited male- and female-phase flowers. Time of measurement, plant, and their interaction were fixed effects; the latter two tested for potential genetic or resource differences among plants. I performed a priori contrasts to compare rates of nectar production between and within male- and female-phase flowers during day, night, morning, and afternoon intervals. All statistical analyses were performed with the Mixed procedure in SAS 9.1 (SAS Institute, Cary, North Carolina, USA), and I included adjustments for heterogeneous variance through time or among seasonal replicates whenever necessary.

I used mass of sugar as the response variable for all nectar analyses; however, nectar volume yielded results very similar to mass of sugar. An average microliter of nectar contained 0.30 mg of sugar (SD = 0.04, N = 306) in unvisited flowers and 0.17 mg of sugar (SD = 0.07, N = 944) in flowers depleted of nectar 2 h prior. In unvisited flowers, sugar content per microliter remained high and constant across volumes, but in depleted flowers, sugar content per microliter increased with volume.

Two-Hour Nectar Replenishment Rates

Nectar replenishment rates were measured in male- and female-phase flowers that were exposed to pollinators, drained, and bagged 2 h before measurement. At the start of each of four 2-h intervals, (0600–0800 hours, 0800–1000 hours, 1000–1200 hours, and 1200–1400 hours), I
selected five plants, each with a male- and a female-phase flower open simultaneously. Flowers were drained by cutting a small flap in the calyx and piercing the back of the corolla, directly above the nectaries. After all nectar was removed using a 10-µL microcapillary and a plastic bulb, the calyx flap was closed and the flower was bagged to prevent pollinator visits during the replenishment interval. At the end of each interval, flowers were collected and the nectar and sugar measured as described. These methods were repeated on at least 11 d during four temporal replicates: late in the 2004 flowering season (September to October), early in 2005 (June to July), and late in 2005 and 2006 (August to September).

In early season 2005, 2-h nectar replenishment was measured in a second set of flowers, to which pollinator visits were experimentally manipulated. These supplementary measurements tested the effects of outcrossed pollen receipt on nectar production in female-phase flowers. Before 0600 hours, 12 pairs of plants were selected, each pair consisting of one plant with two male-phase flowers and one plant with two female-phase flowers. Pollen removal/receipt was experimentally manipulated by inserting a clean *Phaethornis* sp. hummingbird bill into one male-phase flower and immediately inserting it into one female-phase flower on the other plant. The remaining, or control, flower on each plant received no artificial or natural visits. Both the manipulated and control flowers were bagged immediately following treatment application, and bags were only removed briefly when flowers were drained at the start of their 2-h replenishment interval (three plant pairs in each of the four intervals).

Separate statistical models were used to analyze nectar replenishment in flowers with natural pollinator visits and manipulated pollinator visits. For the former, the effects of gender, replenishment interval, temporal replicate, and their interactions on the nectar production rate were tested in a three-way ANOVA. Tukey-Kramer adjustments were used on the least-square (LS) means at each level to compare the differences between and within fixed effects.
For the visit-manipulated subset, nectar replenishment in manipulated and control flowers were compared using a two-way ANOVA on pollination treatment, replenishment interval, and their interaction. Pollination treatment initially had four levels: male-phase flowers with and without pollen removal and female-phase flowers with and without outcrossed pollen donation. Pollen removal did not affect replenishment rate in male-phase flowers ($F_{1,348} = 1.4, P = 0.24$), so pollination treatment was collapsed into three levels: all male-phase flowers, outcrossed female-phase flowers, and control female-phase flowers, which were compared using linear contrasts. The pairing flower and/or plants within experiments was not retained in either analysis, because more than 20% flowers lost their partners to nectar robbers or premature senescence.

**Pollinator Visit Rates**

To measure visit rates of *P. striigularis* to *C. friedrichsthaliana*, two-flowered plants were videorecorded for multiple hours per day. In 2004 and 2005, plants were observed during an early block (June to mid-August) and a late block (mid-August to October). In 2006, plants were observed only during the late block. The sampling methods for each block differed as a result of a marked increase in pollinator visits around mid-August. In the early block, a plant with one male- and one female-phase flower open simultaneously was selected from a randomly chosen subpopulation at 0530 hours, and the plant was recorded with a digital video camera for 10–12 h/d. In the late block, the plant was recorded for only 4 h/d, from 0800 to 1000 hours and then from 1200 to 1400 hours. As I reviewed the video, I counted and timed visits to male- and female-phase flowers for the morning and afternoon of each day of data collection. I also used the 185 d of recording to determine whether and when visits were scarce enough to promote indirect mate competition among plants.
Two separate mixed models were used to test the effects of floral gender, time of day, and time of season on the hourly visit rate and the average visit duration. Gender and time of day were modeled as multivariate repeated measures on plants. Plant and year were random effects.

The Relationships between Pollinator Visits and Male versus Female Fecundity

To determine whether the number of outcrossed pollinator visits differentially affects male versus female fecundity in *C. friedrichsthaliana*, a series of hand pollinations was performed on paired male- and female-phase flowers. On 17 afternoons between June and September 2005, four focal medium-sized plants were selected, such that each would have a male- and a female-phase flower open the following morning. Ten nonfocal plants were selected for mating partners, each with 1–3 incipient male-phase flowers or 1–3 incipient female-phase flowers. The anthers were removed from male-phase flowers to prevent autonomous self-pollination, and all flowers were bagged. The following morning, the bags were removed, and one of four treatments was performed on each focal plant. Both flowers were either (treatment 1) outcrossed with one virgin flower from a nonfocal plant, (treatment 2) outcrossed with two virgin flowers from the same nonfocal plant, (treatment 2D) outcrossed with two virgin flowers from different nonfocal plants, (treatment 3) outcrossed with three virgin flowers from the same nonfocal plant (Figure A.1).

Pollen was transferred between plants using a *Phaethornis* sp. hummingbird bill. Each nonfocal flower was artificially visited only once, but focal flowers of either phase were visited up to three times, depending on the treatment. All female-phase flowers were bagged following pollination, and the bags were removed the next day, at the end of the female phase.

To assess autonomous self-pollination at zero visits, I applied three additional treatments per daily replicate. For the first treatment, I bagged a flower before opening, to prevent all visits
to the male or female phase. For the second, visits were prevented only to the female phase, and natural rates of pollinator visits to the male phase were allowed. For the third, I removed anthers from the flower at the end of the male phase to prevent visits to the female phase. These three treatments estimated fecundity due to maximal, natural, and experimentally reduced self-pollen donation from the male phase, respectively.

Thirty days after the pollination and autogamy treatments were applied, experimental plants were checked daily for open capsules (mean capsule development time = 36.5 d, range = 26–42 d, N = 167). The single capsule produced per flower was collected immediately after dehiscing. After the capsule was dry, seeds were removed, counted, and weighed. *Chrysothemis friedrichsthaliana* seeds are very small and relatively unvarying in mass (1000 seeds = 53 mg, N = 12, SD = 0.008). Seed number was used to estimate fecundity, but total seed mass provided nearly identical results in statistical analyses.

The differences in fecundity of male–female pairs subject to increasing numbers of cross-pollination events was tested using a split-plot ANOVA. Fixed effects were the number of cross-pollination events (treatments 1, 2, 2D, 3), gender, and the interaction. Male- and female-phase flowers were split plots within plants and allowed separate error variances. Plant was a random effect. Linear contrasts were performed to determine whether male and female fecundity increased linearly with cross-pollination events, excluding treatment 2D. Linear contrasts were also used to determine whether seed production in treatment 2 was different from seed production in treatment 2D.

A second ANOVA was performed to compare fecundity of flowers subject to reduced autogamy, natural autogamy, maximum autogamy, and a fourth level comprised of all once-outcrossed male- and female-phase focal flowers. The effectiveness of anther removal was tested by performing a linear contrast of fecundity in the reduced autogamy treatment and in the
natural autogamy treatment. Two additional linear contrasts were performed to determine whether natural or maximal autogamy produced as many seeds as a single outcrossed visit.

RESULTS

Nectar Production Rates

Nectar Accumulation Rates

Unvisited male-phase flowers produced 1.83 times more nectar than unvisited female-phase flowers during daylight hours and 2.91 times more during the preceding night \((P < 0.01)\) for both; Table 3.1). In the morning, nectar production was high and nearly equivalent between phases, but in the afternoon, female-phase flowers produced less nectar than did the male-phase flowers. Afternoon rates were slower than morning rates for both phases. Unvisited female-phase flowers apparently resorbed some nectar during the afternoon, as may be inferred from negative values for production (Table 3.1). Nectar production through time differed significantly among plants (plant: \(F_{15,135} = 9.2, P < 0.0001\); plant \(\times\) measurement time: \(F_{75,135} = 1.6, P = 0.01\)).

Table 3.1 Nectar production in unvisited male- and female-phase flowers of *Chrysothemis friedrichsthaliana* in the Golfo Dulce Forestal Reserve on Osa Peninsula, Puntarenas Province, Costa Rica. Means ± 1 SE are presented for within-gender contrasts, and \(F\) statistics are presented for between-gender contrasts. A statistically significant increase in sugar mass from start to end of an interval is denoted by \(^A P < 0.01\) or \(^B P < 0.0001\). A significant difference in rates between genders is denoted by \(* P < 0.01\) or \(** P < 0.0001\). Numerator degrees of freedom = 1 for all effects; ddf = denominator degrees of freedom.

<table>
<thead>
<tr>
<th>Interval (length)</th>
<th>Within-gender contrasts</th>
<th>Between-gender contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male-phase production (mg sugar)</td>
<td>Female-phase production (mg sugar)</td>
</tr>
<tr>
<td>Preceding night (12 h)</td>
<td>1.86 ± 0.10(^B)</td>
<td>0.64 ± 0.36</td>
</tr>
<tr>
<td>Day (12 h)</td>
<td>3.11 ± 0.20(^B)</td>
<td>1.70 ± 0.44(^A)</td>
</tr>
<tr>
<td>Morning (6 h)</td>
<td>2.68 ± 0.18(^B)</td>
<td>2.45 ± 0.39(^B)</td>
</tr>
<tr>
<td>Afternoon (6 h)</td>
<td>0.43 ± 0.23</td>
<td>−0.75 ± 0.38</td>
</tr>
</tbody>
</table>
Two-Hour Nectar Replenishment Rates

On average, male-phase flowers produced 1.65 times more sugar than female-phase flowers during the 2-h replenishment period \((P < 0.0001; \text{Figure 3.1A, Table 3.2})\). Nectar production in the female phase was greatest during the first two intervals and declined between the second and third \((t_{895} = 4.4, P = 0.0004)\) and third and fourth \((t_{826} = 3.3, P = 0.03)\). In contrast, a reduction in nectar production for male-phase flowers occurred only between the first and fourth \((t_{860} = 4.3, P = 0.0006)\) and second and fourth intervals \((t_{864} = 3.9, P = 0.003)\). By the fourth and final period, male-phase flowers produced 2.89 times as much sugar as female-phase flowers, a 116% increase in the male-to-female ratio from the 0600–0800 hours period.

In all four temporal replicates, male-phase nectar production was higher and declined less rapidly than female-phase production, but total rewards and rate of decline in male and female-phase flowers varied significantly among replicates (Table 3.2). Male- and female-phase flowers produced the most sugar and diverged from one another most over the course of the day in late-season 2004, and they produced the least sugar and diverged least in late-season 2006.

Unvisited, control female-phase flowers produced more sugar than outcrossed female-phase flowers (linear contrast, \(F_{1,100} = 5.1, P = 0.03; \text{Figure 3.1B})\). Regardless of pollination treatment, however, male-phase flowers consistently produced more nectar than female-phase flowers (contrast of outcrossed vs. male-phase, \(F_{1,374} = 30.0, P < 0.0001\); contrast of control vs. male-phase, \(F_{1,374} = 12.4, P = 0.0005\); overall treatment effect, \(F_{2,195} = 15.1, P < 0.0001\)). Nectar production rates decreased throughout the day in all flowers (replenishment interval effect, \(F_{3,285} = 16.7, P < 0.0001\)), but male-phase flowers changed least, and control female-phase flowers changed most (treatment \(\times\) interval, \(F_{6,225} = 3.3, P = 0.004\)).
Figure 3.1 Male bias in 2-h nectar replenishment rates in *Chrysothemis friedrichsthaliana* flowers exposed to (A) natural pollinator visits before bagging and (B) manipulated pollinator visits before bagging in the Golfo Dulce Forestal Reserve on Osa Peninsula, Puntarenas Province, Costa Rica. Least-square means ± 1 SE in (A) are averaged across four replicates: early 2005 and late 2004, 2005, and 2006. Each replicate contributed an average of 33 flowers to each treatment combination (total *N* = 1058). Back-transformed values of square-root least-squares means ± 1 SE in (B) are from early 2005, based on 22–66 flowers per time interval, except the last interval (male, *N* = 42; outcrossed, *N* = 10; control, *N* = 16). Outcrossed female-phase flowers were hand-pollinated once with outcrossed pollen, whereas control female-phase flowers received no outcrossed visits. Average nectar replenishment rate was significantly greater in male-phase relative to female-phase flowers across all four replicates in (A); see Table 3.2 for statistical results. Outcrossed female-phase flowers produced significantly less nectar than controls, and both produced significantly less than male-phase flowers in (B); see Results for details.
Table 3.2 Results of two-way ANOVA of 2-h nectar replenishment rate (mg sugar) of *Chrysothemis friedrichsthaliana* (see also Figure 3.1A). Replenishment interval levels were 0600-0800 hours, 0800-1000 hours, 1000-1200 hours, and 1200-1400 hours. Replicate levels were late-season 2004, early-season 2005, late-season 2005 and late-season 2006. df = numerator degrees of freedom, denominator degrees of freedom.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>1, 884</td>
<td>151.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Replenishment interval</td>
<td>3, 883</td>
<td>34.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Replenishment interval × gender</td>
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<td>4.5</td>
<td>0.004</td>
</tr>
<tr>
<td>Replicate</td>
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<td>84.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Replenishment interval × replicate</td>
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<td>3.3</td>
<td>0.0006</td>
</tr>
<tr>
<td>Gender × replicate</td>
<td>3, 530</td>
<td>0.1</td>
<td>0.94</td>
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<td>Replenishment interval × gender × replicate</td>
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<td>0.12</td>
</tr>
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</table>

**Pollinator Visit Rates**

The hourly visit rate of *P. striigularis* to *C. friedrichsthaliana* plants changed dramatically over the flowering season, yet was fairly consistent for two of three years (Figure 3.2). Hourly visit rate averaged 0.18 ± 0.02 (mean ± SE) during the early block (June to mid-August) and 1.61 ± 0.10 during the late block (mid-August to October). During the early seasons of 2004 and 2005, 17% of monitored plants received one visit per day of recording, and 27% received no visits whatsoever, suggesting strong pollinator limitation in the early season. During the late seasons of all three years, 95% of plants received two or more visits in 4 h.

Overall, male-phase flowers were visited 1.86 times more often than female-phase flowers (*P* < 0.0001, Table 3.3), at 0.76 ± 0.04 visits/h (male-phase LS mean ± SE; Figure 3.3A, B). Visits to male-phase flowers were also significantly longer, at 0.61 ± 0.06 s versus 0.40 ± 0.06 s (Figure 3.3C, D; Table 3.3). For both phases, visit rate and average duration varied significantly across the flowering season, from fewer, longer visits in the early season (Figure 3.3A, C) to many, slightly shorter visits in the late season (Figure 3.3B, D; Table 3.3).
Figure 3.2 Hourly visit rate of *Phaethornis striigularis* to a two-flowered *Chrysothemis friedrichsthaliana* plant between the months June and November 2004 and 2005 and between August and October 2006. Points mark the first day of a 7-d week, over which all days with data were averaged (*N* = 2–7). Weeks started on the same day each year. Error bars represent ±1 SE.

The bias in visit rates toward male-phase flowers was stronger during the late season (*P* < 0.0001, Table 3.3), but the bias in visit duration did not vary across season.

The higher frequency and duration of visits to male-phase flowers became more pronounced in the afternoon relative to the morning (Table 3.3, Figure 3.3A–D), and for visit rate this effect was slightly stronger during the late relative to the early season (e.g., significant three-way interaction for visit rate: *P* < 0.05, Table 3.3). In tests of simple effects, the effect of floral gender was strong in the morning (hourly rate, \( F_{1,91.3} = 46.5, P < 0.0001 \); visit duration, \( F_{1,120} = 5.3, P = 0.02 \)), but even stronger in the afternoon (hourly rate, \( F_{1,144} = 146.2, P < 0.0001 \); visit duration, \( F_{1,147} = 73.3, P < 0.0001 \)).
### Table 3.3
Results of split-block mixed models of hourly visit rate and visit duration of *Phaethornis striigularis* to *Chrysothemis friedrichsthaliana* flowers (see also Figure 3.3). Season levels were early and late. Numerator degrees of freedom = 1 for all effects; ddf = denominator degrees of freedom.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Visit rate (no./h)</th>
<th>Single-visit duration (s)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>ddf</td>
<td>F</td>
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<tr>
<td>Among-plant fixed effect</td>
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<tr>
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<td>Within-plant fixed effects</td>
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<tr>
<td>Gender</td>
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<td>104.5</td>
</tr>
<tr>
<td>Season × gender</td>
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<td>89.5</td>
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<tr>
<td>Time of day</td>
<td>130</td>
<td>26.5</td>
</tr>
<tr>
<td>Gender × time of day</td>
<td>131</td>
<td>7.4</td>
</tr>
<tr>
<td>Season × time of day</td>
<td>130</td>
<td>40.5</td>
</tr>
<tr>
<td>Season × time of day × gender</td>
<td>131</td>
<td>4.0</td>
</tr>
</tbody>
</table>

### The Relationships between Pollinator Visits and Male Versus Female Fecundity

The fecundity of a flower was dependent upon the number of cross-pollination events achieved (*F*<sub>3,37.4</sub> = 8.6, *P* = 0.0002), but the shape of the relationship differed significantly between male- and female-phase flowers (sex, *F*<sub>1,37.2</sub> = 54.7; interaction, *F*<sub>3,37.2</sub> = 14.7, both *P* <0.0001; Figure 3.4). Male- and female-phase flowers that were outcrossed once were similarly fecund; males produced an average of 706.19 ± 276.9 seeds (±SE) and females produced an average of 818.88 ± 79.43 seeds. As the number of cross-pollination events increased, however, significant differences between male and female fecundity emerged. Male fecundity increased linearly from one to three cross-pollination events (excluding treatment 2D; *F*<sub>1,29</sub> = 54.1, *P* < 0.0001), whereas female fecundity decreased slightly across the same treatments (*F*<sub>1,29</sub> = 6.8, *P* = 0.01). Fecundity did not differ between flowers that were outcrossed with two partners from different plants relative to two partners from the same plant, for male-phase flowers (*F*<sub>1,37</sub> = 0.5, *P* = 0.48) and female-phase flowers (*F*<sub>1,37</sub> = 0.09, *P* = 0.76).
Figure 3.3 Response of *Phaethornis striigularis* to male- and female-phase *Chrysothemis friedrichsthaliana* flowers in the morning and afternoon. Pollinator response measured as number of visits to individual flowers per hour in the (A) early season and (B) late season and the duration of flower visits in the (C) early season and (D) late season. Data are averaged over three years. Early season data consist of 10–12 h observation periods per day (\(N=82\)). Late-season data consist of 2 h in the morning and 2 h in the afternoon (\(N=103\)). Means are least squares, and error bars represent ±1 SE. Average hourly visit rate and duration of single visits were significantly greater for male- relative to female-phase flowers in the morning and afternoon and in the early and late seasons; see Table 3.3 for statistical results.

Seed production in unvisited flowers varied with the degree of self-pollen donation (overall ANOVA, \(F_{3,43.3}=10.7, P<0.0001\); Figure 3.5). Seed production following anther removal was not significantly lower than seed production following natural levels of autogamy (\(F_{1,33.1}=0.7, P=0.40\)). Flowers restricted to natural levels of autogamy were, however, only half as fecund at those exposed to a single outcrossed visit (\(F_{1,50.3}=17.9, P<0.0001\)). In contrast to other autogamy treatments, seed production following maximum autogamy was similar to that of a single outcrossed visit (\(F_{1,37.9}=2.3, P=0.13\)).
Figure 3.4  Relationships between outcrossed pollination success and male and female fecundity in *Chrysothemis friedrichsthaliana*, in terms of number of seeds produced. Means are least squares, and error bars represent ±1 SE. Treatments were numbers of cross-pollination events per focal flower. Treatment 2 used two mates from the same plant, treatment 2D used two mates from different plants, and treatment 3 used three mates from the same plant. Sample sizes, in order of appearance on graph, were 10, 11, 9, and 11 male–female pairs. Fecundity of male-phase flowers increased linearly with number of cross-pollination events, excluding treatment 2D, whereas fecundity of female-phase flowers did not; see Results for details.

Figure 3.5  Autogamous seed production of unvisited *Chrysothemis friedrichsthaliana* flowers subject to varying degrees of self-pollen donation. Means are least squares, and error bars represent ±1 SE. Sample sizes of bars, in order of appearance on graph, were 11, 11, and 22 flowers. Heavy dotted line represents mean seed production of male- and female-phase flowers that received a single cross-pollination event (*N* = 20), and light dotted lines are ±1 SE. Natural autogamy produced significantly fewer seeds than outcrossing, but maximal autogamy and cross-pollination produced similar numbers of seeds; see Results for details.
DISCUSSION

In *C. friedrichsthaliana*, the nectar production pattern, pollinator response, and gender-specific pollination needs readily align with four key sexual selection predictions. An adaptive interpretation of male-biased nectar production in *C. friedrichsthaliana* is supported by (1) the unambiguous male bias in nectar production; (2) pollinator limitation in the early flowering season; (3) a bias in hummingbird visits toward male-phase flowers, potentially in response to nectar; and (4) the saturation of female, but not male, function after one pollinator visit or autonomous self-pollination. Next, I summarize and relate findings to predictions from sexual selection theory.

**Male-Biased Nectar Production Persists Through Time**

*Chrysothemis friedrichsthaliana* produced more nectar in male-phase flowers across three years and under a diversity of conditions, which meets the first requisite of an interpretation involving sexual selection. A male bias was observed across changing patterns of precipitation within and between seasons (J. E. Carlson, unpublished data) and under different experimentally imposed pollination and nectar depletion regimes.

Nectar production was nevertheless dynamic in *C. friedrichsthaliana*, and the strength of bias varied with time and pollinator visits. For example, both male- and female-phase flowers produced less nectar as the day progressed, but daily reductions were greater during the female phase. As a result, the male bias in nectar production strengthened from morning to afternoon. An increase in male bias over the course of the day has not been widely documented (Cruden et al., 1983; Rivera et al., 1996), but it is not unusual for plants to reduce nectar production throughout the day (e.g., Stiles, 1975; Cruden et al., 1983; McDade and Weeks, 2004).

Nectar production also responded directly to both pollen delivery and nectar removal. Cross-pollination reduced female-phase nectar production in the early morning, although
differences between outcrossed and control flowers disappeared by 1000 hours. Comparisons of nectar accumulation versus net short-term replenishment suggest that nectar production in *C. friedrichsthaliana* is further reduced by nectar depletion. Nectar-depleted, unvisited male- and female-phase flowers produced less sugar and differentiated more throughout the morning, relative to undepleted, unvisited flowers. Assuming a minimal effect of the different techniques and plants used, this indicates that depletion, like pollen delivery, strengthens the male bias in nectar production. A decrease in nectar production in response to depletion has been observed in a few additional species (e.g., Cruden et al., 1983; McDade and Weeks, 2004), although the opposite trend, a positive response to depletion, appears to be more commonly observed (Ordano and Ornelas, 2004; reviewed in Castellanos et al., 2002). Ordano and Ornelas (2004) and Aizen and Basilio (1998) found no effects of pollen delivery on nectar production in simultaneously hermaphroditic and protandrous plant species, respectively. Further investigation into the relative importance and adaptive value of the nectar regulation mechanisms of *C. friedrichsthaliana* and other hermaphroditic species is clearly warranted (e.g., Castellanos et al., 2002).

The constancy of male-biased nectar production in *C. friedrichsthaliana* suggests a genetic basis for the trait. No study has directly examined the heritability of male-biased nectar production, although at least six studies show nectar production rates themselves can be highly heritable, even in the face of strong environmental effects (e.g., Mitchell and Shaw, 1993; Boose, 1997; Klinkhamer and van der Veen-van Wijk, 1999; reviewed in Mitchell, 2004). In *C. friedrichsthaliana*, the significant effect of the source plant on nectar accumulation indicates that there is genetic variation in nectar accumulation rates, and additional preliminary work has shown genetic variation in the sex bias (Chapter 1). Thus, selection mediated by pollinators
could influence (or influenced historically) the frequency of male-biased nectar production in the population.

**Pollinator Visits May Be Limiting in Early Season**

*Phaethornis striigularis* visited single plants fewer than two times per day during most of the early season, and this number remained low until after peak flowering for both years. Only as flowering rate declined did the per-plant visit rate begin to increase. Other studies documenting hummingbird visits through time also find that they fluctuate with season (Wolf, 1970; Stiles, 1975; Feinsinger, 1976; Waser and McRobert, 1998), and in *C. friedrichsthaliana*, these changes in visit rate through time create an environment in which pollinator visits are limiting.

Pollinator limitation is a second requisite of trait maintenance through sexual selection for increased visit quantity (although not increased visit quality). Sexual selection implies that plants indirectly compete for pollinators, which can only occur when there are not enough pollinator visits to meet the reproductive needs of all plants (Haig and Westoby, 1988; Burd, 1994). Between June and mid-August, each male-phase and female-phase flower produced by a *C. friedrichsthaliana* plant has a 17% chance of receiving one visit and a 27% chance of receiving zero visits, based on two years of observation. Flower production reaches its peak during this time, suggesting that pollination limitation occurs at a crucial portion of the plant’s reproductive cycle. If male- and female-phase flowers are indeed differentially limited by visits, pollinator limitation may promote sexual selection on nectar production.

**Pollinators Respond to Nectar with Increased Male-Phase Visits**

Pollinators preferentially visited male- over female-phase flowers, which I interpret as a direct response to the nectar they provided. Multiple lines of evidence suggest that differential nectar ultimately underlies male-biased pollinator visit rates in *C. friedrichsthaliana*. First,
male- and female-phase flowers do not differ notably in other secondary traits that may influence preferences independent of nectar, such as color (e.g., Melendez-Ackerman and Campbell, 1998) or nectar accessibility to pollinators (e.g., Temeles et al., 2002). Second, hummingbirds are known to perceive and respond directly to nectar in many plant species, and they readily discriminate against less-rewarding flower types as they occur in both space and time (Stiles, 1975; Best and Bierzychudek, 1982; Gass and Sutherland, 1985; Hurly, 1996; Aizen and Basilio, 1998; Healy and Hurly, 2001).

In *C. friedrichsthaliana*, evidence that birds are responding directly to nectar is circumstantial and requires further investigation, but current findings are nevertheless compelling. Visit frequencies and durations decrease over the course of the day, as do nectar production rates in male- and female-phase flowers. Additionally, the ratio of pollinator visits to male- relative to female-phase flowers, 1.86, is close to the ratio of the 2-h nectar replenishment rate in male- relative to female-phase flowers, 1.65. Two other studies have found that pollinators also tracked nectar production rates in plants with male-biased nectar production (Bell et al., 1984; Aizen and Basilio, 1998), although in neither are the ratios as similar as that found here. Clearly, rates and responses need not match exactly for nectar to have higher benefit to male-phase flowers, but in the case of *C. friedrichsthaliana*, they are surprisingly similar.

The matching of visits to nectar production meets a third requisite of sexual selection in plants. Extra nectar in male-phase flowers increases the probability of multiple male-phase visits during the early season, relative to female-phase visits. During the late season, selection could act through the quality of pollinator visits, rather than the quantity, because visits are not limiting at that time. In sum, the current-day behaviors of pollinators at *C. friedrichsthaliana* plants are generally consistent with those predicted by sexual selection theory to maintain male-biased nectar production.
Increased Pollinator Visits Benefit Male More Than Female Fecundity

In *C. friedrichsthaliana*, an increase in pollinator visits from one to three clearly benefited male fecundity more than female fecundity. These results align *C. friedrichsthaliana* with one of the most basic predictions of sexual selection theory: male mating success has a stronger linear relationship with fecundity than does female mating success (Bateman, 1948). Stronger pollinator limitation on male fecundity has been documented in numerous species (e.g., Bell, 1985; Willson, 1994; Queller, 1997), but is not always observed (Broyles and Wyatt, 1990, 1995). The frequent occurrence of pollen limitation on female fecundity (e.g., Burd, 1994; Ashman et al., 2004) suggests that stronger intra-male competition for visits cannot be assumed, yet it is still likely in many plant species. In *C. friedrichsthaliana*, male fecundity is more strongly pollinator limited than female fecundity most prominently in the first half of the flowering season, when visit rates are low and receptive stigmas are abundant throughout the day.

Female-phase flowers were able to produce some seeds in the absence of pollinator visits, through autonomous self-pollination. When all male-phase and female-phase visits were prevented, flowers were capable of producing a full set of seeds, which maximized female but not male fecundity. Reproductive assurance through autogamy essentially guarantees seed production when pollinators are scarce (Jarne and Charlesworth, 1993), which is a characteristic of the early season for *C. friedrichsthaliana*. Autogamy may nevertheless incur costs through inbreeding depression (Charlesworth and Charlesworth, 1987), pollen discounting (e.g., Fishman, 2000), and seed discounting (Herlihy and Eckert, 2002), although inbreeding depression does not appear to be particularly high in *C. friedrichsthaliana* (Chapter 4). A high frequency of autogamy and a potentially low cost of inbreeding suggest that pollen recipients are very rarely limited by access to mates in this species. Thus, females are unlikely to experience
strong selection to better compete for pollinator visits, although males almost certainly will.

Given these clearly divergent relationships between fecundity and pollination success, in addition to the described evidence, it stands to reason that sexual selection contributes strongly to the maintenance of male-biased nectar production in *C. friedrichsthaliana*. To the degree that current-day patterns approximate those important on evolutionary timescales, pollinator-mediated sexual selection may have been a driving force in the evolution of male-biased nectar production in this and, quite likely, other hermaphroditic plants.

**LITERATURE CITED**


Chapter 4

Hummingbird Responses to Within-Plant Variability in Nectar: Tests of the Sexual Selection and Inbreeding Avoidance Hypotheses

INTRODUCTION

Many plant species depend on nectar-seeking animals to outcross, yet their per-flower nectar production rates differ from those preferred by pollinators. Pollinators make more and longer visits to plants with relatively high nectar production, which may confer higher plant fitness (e.g., Real and Rathcke, 1991; Mitchell, 1993; Schemske and Bradshaw, 1999). Nevertheless, variable nectar production among flowers on the same plant is commonly observed (e.g., Cruden et al., 1983; Boose, 1997; Cresswell, 1998; McDade and Weeks, 2004; Herrera et al., 2006). For example, at least 41 plant species in 18 families exhibit gender-biased nectar production, a pattern that results in variable rewards within multi-flower bisexual displays (Carlson and Harms, 2006). In these species, within-plant variability occurs because dichogamous flowers increase or decrease nectar production through time, as each flower passes between male and female floral phases. Nectar production rates are higher during the male phase in at least 21 species and higher during the female phase in at least 20 species (Carlson and Harms, 2006).

Gender-biased nectar production and other patterns of within-plant variability may confer a selective advantage over high, constant rewards under certain conditions (Pleasants, 1983; Bell et al., 1984; Rathcke, 1992). Even so, the adaptive benefits of variable rewards have rarely been tested or demonstrated (Biernaskie et al., 2002). Carlson and Harms (2006) outline two main mechanistic explanations for gender-biased nectar production: the sexual selection hypothesis,
based on sexual selection theory, and the inbreeding avoidance hypothesis, based on costs of inbreeding and pollinator foraging theory.

The sexual selection hypothesis postulates that plants temporally adjust nectar production within flowers to match the differential nectar-fitness functions of each floral phase. Male fecundity often (but not always) requires more pollinator visits to be maximized than does female fecundity, since ovules and offspring are relatively few and expensive to produce, whereas pollen grains are inexpensive and plentiful (Bateman, 1948; Trivers, 1972; Arnold, 1994). A stronger relationship between nectar and male fitness should favor male-biased nectar production if pollinators preferentially visit more-rewarding flowers.

The inbreeding avoidance hypothesis, in contrast, predicts that plants with variable rewards experience lower rates of inbreeding between flowers on the same plant, i.e., geitonogamy, relative to plants with high, constant rewards (Pleasants, 1983; Rathcke, 1992; de Jong, et al., 1993; Carlson and Harms, 2006). This is because according to foraging theory, pollinators should respond to variable rewards with behaviors that potentially reduce geitonogamy. Namely, they should move from high to low rewards within plants, avoid less-rewarding options altogether (Pyke, 1978; Stephens and Krebs, 1986), or sample fewer flowers per plant when rewards are unpredictable (e.g., risk averse behavior; Kacelnik and Bateson, 1996; Biernaskie et al., 2002; Hirabayashi et al., 2006). Any of these responses may favor variable rewards when inbreeding is costly (e.g., Charlesworth and Charlesworth, 1987; de Jong et al., 1993; Eckert, 2000).

Together, the sexual selection and inbreeding avoidance hypotheses are equipped to describe the ecological maintenance of certain patterns of within-plant variability in nectar. Recent work has focused on gender-biased nectar production in the dichogamous herb *Chrysothemis friedrichsthaliana* (Gesneriaceae), which is pollinated by the hermit hummingbird.
Phaethornis striigularis saturatus (Trochilidae: Phaethornithinae, Figure 4.1). In a three year study, Carlson (2007) provided support for four sexual selection predictions regarding variable nectar in *C. friedrichsthaliana*. First, nectar production rates were consistently higher during the male as opposed to female floral phase. Second, *P. striigularis* spent 53% more time per visit and made 86% more visits to male- compared to female-phase flowers. Third, female fecundity was maximized by one outcrossed visit, whereas male fecundity continued to increase up to at least three visits. Female fecundity was near-saturated by autogamous seed production, which results from self-fertilization within flowers. Even so, an outcrossed visit achieved higher seed production than natural levels of autogamy. A fourth prediction met was that pollinator visits were at least occasionally in short supply. Based on these findings, Carlson (2007) concluded

**Figure 4.1** *Phaethornis striigularis* with a real *Chrysothemis friedrichsthaliana* flower and an artificial flower, both of which were used in nectar manipulation experiments. Line drawing by Jane E. Carlson.
that sexual selection may at least partially explain male-biased nectar production in \textit{C. friedrichsthaliana}. Nevertheless, a final prediction remains untested – that pollinator preferences are in direct response to nectar.

The inbreeding avoidance hypothesis may also be relevant for \textit{C. friedrichsthaliana}. \textit{Chrysothemis friedrichsthaliana}’s small floral displays include multiple flowers (typically two) and both phases approximately 33\% of the time when flowering (Chapter 1, Figure 1.4). Furthermore, \textit{P. striigularis} hummingbirds move from the male- to the female-phase flower within a two-flowered plant on roughly one-third of their visits. Given that geitonogamous inbreeding is possible in \textit{C. friedrichsthaliana}, the following predictions of the inbreeding avoidance hypothesis warrant testing: pollinators should depart sooner from plants with variable versus constant rewards, responses should be based predominately on nectar, and within-plant pollen movement should decrease plant fitness.

In this study, I used experimental manipulations of nectar and pollination in \textit{C. friedrichsthaliana} to assess the adaptive functions of variable nectar, in terms of sexual selection and inbreeding avoidance. First, I exposed \textit{P. striigularis} to pairs of real male- and female-phase flowers with altered nectar production patterns, and I measured hummingbird responses. Second, I measured \textit{P. striigularis} responses to nectar in pairs of artificial flowers that had no gender-specific floral cues. Third, I used hand pollinations to assess the costs of inbreeding, in terms of seed production and quality.

I compared experimental results to a key remaining prediction of the sexual selection hypothesis and two predictions of the inbreeding avoidance hypothesis. To support the sexual selection hypothesis, \textit{P. striigularis} should respond directly to nectar and therefore preferentially visit the more-rewarding flower in pairs of real or artificial flowers. To support the inbreeding avoidance hypothesis, the following two predictions should be met: (1) \textit{Phaethornis striigularis}
should respond to within-plant reward variability by making more single- as opposed to multiple-flower visits to plants with real flowers. When visiting plants with artificial flowers, they should respond to variable rewards by making more single-flower visits or by making more high- to low-reward moves within plants; and (2) there should be reproductive costs of geitonogamous inbreeding in *C. friedrichsthaliana*, such as reduced seed number, weight, germination, or viability.

**MATERIALS AND METHODS**

**Species and Study Site**

I studied *C. friedrichsthaliana* on the property of the Fundacion Neotrópica’s Centro Tropical, in Agua Buena de Rincón, Osa Peninsula, Costa Rica. Plants in this population are pollinated almost exclusively by *P. striigularis* (Figure 4.1; Chapter 1, Table 1.1), a small (2.6 g), non-territorial, traplining, hermit hummingbird of Neotropical lowland forests (Skutch, 1951; Stiles and Skutch, 1990; Hinkelmann and Schuchmann, 1997). Their 2.2 cm long bills (SD = 0.07 N = 9; J. E. Carlson unpublished data) enable them to access *C. friedrichsthaliana* nectar, which is produced by nectaries at the base of a 2.56 cm (SD = 0.13, N = 49), orange, tubular corolla. In 2004 and 2005, at least 17 different *P. striigularis* individuals visited plants at Centro Tropical and apparently relied heavily on flowers of *C. friedrichsthaliana* when they were available (J. E. Carlson, personal observation).

The two-day, protandrous flowers of *C. friedrichsthaliana* decrease nectar production rates between and within sexual phases (Carlson 2007). The more-rewarding male phase precedes the female phase in time, and each phase lasts an average of 12 daylight hours. Male-phase flowers produce roughly 20% more nectar in the early morning and 50% more nectar in the early afternoon, relative to female-phase flowers (mean ± s.e. of 2-h replenishment rate of male phase in early morning: 2.45 ± 0.02 μL nectar or 0.46 ± 0.07 mg sugar; female-phase in
early morning $2.04 \pm 0.02 \mu L$ or $0.34 \pm 0.07$ mg sugar; J. Carlson, unpublished data; Carlson 2007). When nectar production is at its peak in either phase, unvisited flowers contain an average of $8-9 \mu L$ nectar and $2.5-2.7$ mg sugar (Carlson 2007). Some autogamous self-fertilization occurs within flowers as they transition between phases (i.e., anthers curl back and stigma grows forward). In flowers unvisited during either phase, $617 \pm 63$ seeds (mean ± SE) may be produced via autogamy, which rivals seed production following a single outcrossed visit to a female-phase flower on a small plant ($754 \pm 64$; Carlson, 2007).

*Chrysothemis friedrichsthaliana* is perennial and flowers from May to November, with peak flowering in July or August, at 1 flower per day (Skutch, 1992; Chapter 1, Figure 1.3). I carried out experiments from August to November in 2004, 2005 and 2006, which corresponded to the second half of *C. friedrichsthaliana*’s flowering season and the peak of the rainy season.

**Pollinator Responses to Nectar in Real Flowers**

I used natural patches of *C. friedrichsthaliana* plants to test the responses of *P. striigularis* to male-biased, female-biased, and unbiased nectar production in real male- and female-phase flowers. At the outset of the experiment, I selected three large plants growing in close proximity. I manipulated and observed these as a patch for three consecutive weeks (Appendix B, Figure A.2). On one day each week, I applied a different nectar treatment to each plant, followed by a two-hour observation period. By the end of three weeks, all plants had received all treatments in a Latin square design (Appendix B, Figure A.2). I replicated this design in four patches in 2004 and 2005, and three in 2006. Patches studied within the same year were $\geq 50$ m apart, were on the traplines of different hummingbirds, and were observed on different days.

To apply nectar treatments to plants, I arrived to a patch before sunrise (~0500 hours, local time) and adjusted each floral display to exactly one male- and one female-phase flower. I
drained the standing crop of nectar from all six flowers, using a 10-µL microcapillary tube pierced through the back of the corolla. I used the same hole to immediately pipette small volumes of 25% sucrose solution back into the flower, according to nectar treatments. In the male-biased treatment, female-phase flowers received no nectar and were re-drained at 40 and 80 min. Male-phase flowers received 10 µL nectar (2.75 mg sugar) at the start of the experiment, and they were drained and refilled with 4 µL (0.83 mg) at 40 and 80 min. In the female-biased treatment, the pattern applied in the male-biased treatment was reversed between male- and female-phase flowers. In the unbiased treatment, both flowers first received 5 µL (1.38 mg) and were drained and refilled with 2 µL (0.55 mg) at 40 and 80 min. I selected experimental nectar volumes to span the range of nectar volumes in unvisited flowers in the morning, when nectar production is highest (Carlson, 2007; Chapter 1, Figure 1.7). These were exaggerated slightly to overwhelm the small volumes of nectar produced naturally by the nectar-manipulated flowers. I refilled flowers at 40 and 80 minutes with lesser volumes to mimic natural reward reduction following a flower’s first visit.

Starting at sunrise (0530 hours, local time), I used a digital video camera to record two consecutive hours of hummingbird activity at the nectar-manipulated patch. I reviewed hummingbird visits in slow motion (1/3 speed) and recorded the following: the number of visits to each flower and each plant, the length of time the bill was inserted into a flower, and the order of visits within plants. I used these data to calculate 2-h visit rate, single-visit duration, and the proportions of each type of visit made to plants. Phaethornis striigularis visited plants in one of three ways (visit types): they visited only one flower, they visited both flowers in order of male-to female-phase, or they visited both flowers in order of female- to male-phase.

I performed statistical tests on three aspects of P. striigularis visits to determine how they responded to nectar rewards within plants and flowers. First, I tested how P. striigularis altered
2-h visit rates to flowers in response to nectar treatment, floral gender, patch of plants, and all fixed-effect interactions. I used a generalized linear mixed model with a Poisson distribution and heterogeneous variance across years. The experimental design included a split-plot of floral gender within plants, which accounted for non-independence of flowers from the same plant. Plants were in a Latin square with week and nectar treatment. Second, I tested how *P. striigularis* altered single-visit durations to flowers in response to the same fixed effects, using the same experimental design as for the first test. Third, I tested how the probabilities of each of three visit types changed in response to nectar treatments, using the following modification to the above design. I changed the distribution to generalized multinomial, and I set single-flower visits as the reference category (SAS Institute Inc., 2005). The only fixed effect was nectar treatment, and random effects were patch of plants, plant, week, and year. I compared the probabilities of each visit type among nectar treatments using three linear contrasts for male-then-female visits, and three linear contrasts for female-then-male visits. Comparisons for single-flower visits were unnecessary, because all three visit types sum to 100%. Unless otherwise specified, I performed all statistical analyses using the Glimmix procedure in SAS 9.1.3 (Cary, North Carolina, USA).

**Pollinator Responses to Nectar in Artificial Flowers**

I used arrays of artificial flowers to test *P. striigularis* responses to varying nectar production patterns in the absence of gender-specific floral cues. I constructed artificial flowers using 10 µL pipettor tips, sealed at the tip, painted, and bordered with orange and red plastic corollas, to resemble real *C. friedrichsthaliana* flowers (Figure 4.1). I attached 16 artificial flowers to eight live, potted *C. friedrichsthaliana* plants, two per plant (Appendix A, Figure A.3). I arranged the plants on a low, inconspicuous table on the foraging route of a targeted hummingbird. To encourage the hummingbird to revisit artificial flowers, I added 6 µL 25%
sucrose solution (1.65 mg sugar) to all flowers on an hourly basis for several days, until the start of the experiment.

Once a single, marked hummingbird was frequently visiting an array, I started applying nectar treatments. Starting at 0800 hours on each of 8 days, I added to every flower either 0, 2, 3, 4, or 6 µL of 25% sucrose solution (0, 0.55, 1.10, and 1.65 mg sugar, respectively). I refilled flowers with the same nectar volume hourly until 1500 hours, and the next day, I applied new nectar volumes to flowers. I assigned nectar volumes on a per-plant basis in six unique nectar production patterns, or treatments, divided between two experiments on different arrays.

In one experiment, I created variable rewards between artificial flowers on plants in four different patterns. I selected nectar production patterns that were analogous to varying degrees of gender-biased nectar production in real *C. friedrichsthaliana* flowers, at the high end of the natural range nectar volumes and sugar contents of unmanipulated flowers (Chapter 1, Figure 1.7). These patterns included 2 µL to the flower on the left and 0 µL to the flower on the right (e.g., 2-0 µL), 4-0 µL, 4-2 µL, 6-2 µL. For the remaining four plants, I exchanged volumes between left and right flowers (Appendix; Figure A.3). I performed a second experiment on the same hummingbird after the first experiment was completed. I removed two plants from the array and applied the following treatments to each of six plants: 6-0 µL, 3-3 µL, 4-2 µL. For the remaining three plants, I reversed the order of treatments between flowers. By the end of each eight or six day experiment, I had assigned all eight or six treatments to each plant, in a Latin square design. In analyses, I combined treatment pairs that assigned the same nectar volumes in reverse order (e.g., 0-2 and 2-0 µL).

Starting at 1400 and 1500 hours daily, I observed two foraging bouts to the array, and I refilled flowers between bouts. I recorded which plants and flowers hummingbirds visited and the order of visits into a hand-held tape recorder. Only the first hummingbird visit to each plant
was counted, and each flower was visited no more than once per foraging bout. To calculate visit probabilities, I coded each foraging bout as a series of 16 (or 12, for the second experiment) successes and failures to visit individual flowers within plants. In the first experiment, I observed a total of 32 bouts per treatment (16 bouts per plant) for each hummingbird, and in the second experiment I observed 24 bouts per treatment (12 bouts per plant) for each hummingbird. To calculate percentages of each visit type, I again assigned hummingbird visits to one of three visit types: they visited one of the two flowers, they visited both flowers in order of higher to lower rewards, or they visited both flowers in order of lower to higher rewards. Treatment 3-3 had only two visit types: they visited only one flower or they visited both flowers.

Between August and October of 2005 and 2006, I observed five different hummingbirds visit the array for all eight days in the first experiment. Only two individuals continued visiting for the six additional days needed to complete the second experiment. These two hummingbirds behaved similarly when foraging in arrays with plants providing equal total volumes (i.e., experiment two) as compared to arrays with plants providing different total volumes (i.e., experiment one). When the foraging responses of the two hummingbirds were tested in preliminary analyses, there was no significant effect of experiment one versus two ($F < 0.6$, $P > 0.4$ for both visit probabilities and types). Given the reduced sample size of the second experiment and its similarity with the first, I combined the two experiments in final analyses.

I used three logistic regressions to test pollinator responses in the absence of floral cues. First, I used a binary logistic regression to test whether the probability of a visit was dependent on a flower’s nectar volume (0, 2, 3, 4, 6 µL nectar), the volume of its companion flower, or the specific treatment combination of the plant (2-0, 4-0, 6-0, 3-3, 4-2, or 6-2 µL nectar). The hummingbird’s identity was an additional fixed effect, as was its interaction with a flower’s nectar volume. Plant and day were random effects nested within hummingbird in a Latin square.
The two flowers per plant were modelled as a split plot on plants, as were the two foraging bouts per day. Second, I used a multinomial logistic regression to test whether the probability a plant of receiving any visit type was dependent on the nectar treatment or hummingbird, excluding the 3-3 µL treatment (SAS Institute Inc., 2005). I modeled random effects as above, using a generalized multinomial distribution (reference category=single-flower visits). I used linear contrasts to compare the probabilities of receiving each visit type among treatments, including contrasts of plants with an unrewarding flower against those without an unrewarding flower. Third, I added the 3-3 µL treatment to the preceding analysis to compare pollinator responses to constant rewards within plants versus other nectar treatments. I used the same fixed and random effects as in the preceding regression, but I changed the distribution to binary and I re-categorized all visits into two types: a single- or a two- flower visit. Linear contrasts compared the probability of a two-flower visit to plants in treatment 3-3 µL versus plants in treatment 6-0 and 2-4 µL.

Costs of Inbreeding

To determine if inbred \textit{C. friedrichsthaliana} flowers produced seeds of lower qualities or quantities than outcrossed flowers, I hand-pollinated flowers and tested germination and viability of the resulting seeds. Twice each week during an 8-week period in 2004 and again in 2005, I selected two large, robust plants per day, each with at least two male-phase flowers and at least two mature buds. I left male-phase flowers on selected plants open to pollinator visits until the late afternoon, which removed most available pollen and approximated anther removal at the end of the male-phase (Carlson, 2007; J. E. Carlson, unpublished data). The evening before treatments were applied, I bagged male-phase flowers and randomly assigned each a hand-pollination treatment. Both hand-pollination treatments were represented within a plant and consisted of either two inbred visits, each from a different previously unvisited male-phase
flower on the same plant (inbred); or two outcrossed visits, each from a different unvisited male-phase flower on a neighboring plant (outcrossed). For each treatment, a clean *Phaethornis* sp. bill was inserted once into a donor male-phase flower and then once into the recipient female-phase flower. The bill was then cleaned with alcohol and the steps were repeated using the other male-phase flower, to approximate two *P. striigularis* visits with maximum pollen loads. After applying the treatments, flowers were bagged until the corolla detached the next day. Capsules were collected immediately after they dehisced (~35 days later), and after they dried, seeds were removed and stored.

Germination trials began roughly five months after capsules were collected in 2004 and 2005, to mimic the natural period of dormancy of *C. friedrichsthaliana* seeds (Skutch, 1992). Seeds were weighed and counted, and from each capsule, a random subsample of 100 seeds was placed into a petri plate on moistened Whatman #10 filter paper. Plates were placed in clear plastic containers lined with moistened paper towels, and these were kept in an incubator for 23 days at 26°C and 55% humidity with a 12 h d and 12 h night. I checked for germinated seeds and rearranged plates within the incubator every other day. At the end of the 23 d trial, I tested ungerminated seeds for potential viability by applying pressure to the seed and checking for emergence of firm, white endosperm. For each capsule, I recorded the number of seeds produced, the average mass per seed, the percent of seeds germinated and the percent of seeds viable (including those germinated) after 23 days.

I performed four 2-way ANOVAs to test the effects of inbreeding versus outcrossing on four variables representing seed production and quality in *C. friedrichsthaliana*. Two response variables, percent seeds germinated and percent seeds viable, were logit transformed and analyzed using generalized linear mixed models with binomial probability distributions. The remaining two variables, seed count per flower and seed mass, did not violate assumptions of
normality and were therefore analyzed using mixed linear models (Mixed Procedure, SAS 9.1.3).

Since I replicated the experiment once per year for only two years, I included year as an additional fixed effect, as was its interaction with hand-pollination-treatment. In all four analyses, plant was a random effect accounting for paired treatments within plants; however, a few plants experienced herbivory and did not contain both treatments by the experiment’s end.

**RESULTS**

**Pollinator Responses to Nectar in Real Flowers**

*Phaethornis striigularis* altered visit rates and durations as nectar volumes increased, but their responses differed between male- and female-phase flowers (Table 4.1; Figure 4.2 A,B).

**Table 4.1** Results of split-block generalized mixed linear model of 2-h visit rate and single-visit duration of *Phaethornis striigularis* at nectar manipulated, two-flowered *Chrysothemis friedrichsthaliana* plants in the Golfo Dulce Forestal Reserve on Osa Peninsula, Puntarenas Province, Costa Rica (see also Figure 4.2). Nectar treatments were male-biased, female-biased, and unbiased production within plants. A patch was three adjacent plants on a hummingbird’s foraging route, during a given flowering season. Flowering season was a random effect, grouping the 3 replicates (~3 hummingbirds) in 2006 and the 4 replicates in each 2004 and 2005. Ndf = numerator degrees of freedom; ddf = denominator degrees of freedom.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Visit rate (no./2-h)</th>
<th>Single-visit duration (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ndf  ddf  F   P</td>
<td>ndf  ddf  F   P</td>
</tr>
<tr>
<td>Among-plant fixed effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>2     58.9 1.1 0.35</td>
<td>2    76.8 2.6 0.079</td>
</tr>
<tr>
<td>Patch</td>
<td>10    40.7 5.3 &lt;0.0001</td>
<td>10   24.4 38.8 &lt;0.0001</td>
</tr>
<tr>
<td>Patch × Treatment</td>
<td>20    44.0 0.6 0.92</td>
<td>20   25.5 1.1 0.45</td>
</tr>
<tr>
<td>Within-plant fixed effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>1     85.8 51.3 &lt;0.0001</td>
<td>1   110.4 20.5 &lt;0.0001</td>
</tr>
<tr>
<td>Treatment × Gender</td>
<td>2     85.7 11.5 &lt;0.0001</td>
<td>2   110.4 37.3 &lt;0.0001</td>
</tr>
<tr>
<td>Patch × Gender</td>
<td>10    45.5 2.1 0.041</td>
<td>10   54.1 1.7 0.12</td>
</tr>
<tr>
<td>Treatment × Gender × Patch</td>
<td>20    46.0 0.8 0.73</td>
<td>20   54.9 1.1 0.37</td>
</tr>
</tbody>
</table>
Figure 4.2 Visit rate (A) and average single-visit duration (B) of *Phaethornis striigularis* at nectar-manipulated *Chrysothemis friedrichsthaliana* plants in the Golfo Dulce Forestal Reserve on the Osa Peninsula, Puntarenas Province, Costa Rica. Between August 2004 and October 2006, 33 different plants were observed three times each, once per nectar treatment. Least square means with different lowercase letters are significantly different (Tukey-Kramer adjusted, $\alpha = 0.05$), and error bars represent ± 1 SE. See Table 4.1 for complete statistical results.
Female-phase flowers with more nectar (i.e., those in the female-biased treatment) received relatively more and longer visits than those with less nectar, indicating that hummingbirds responded positively to nectar in female-phase flowers (Figure 4.2 A,B). Even so, hummingbirds visited male-phase flowers more often than female-phase flowers, independent of whole-plant nectar treatment (Table 4.1). Male-phase flowers with more nectar were visited at the same, relatively high rate as those with less nectar (Figure 4.2 A), but visit duration to male-phase flowers increased with nectar rewards (Figure 4.2 B).

Hummingbird foraging behavior was variable among the 11 plant patches. Individual birds and/or the spatial location and arrangement of each patch had a significant effect on visit rates and durations (Table 4.1). Even so, preferences for nectar treatments and floral genders were generally consistent across patches (i.e., 2- and 3-way interactions with patch of plants were non-significant, except for the patch × gender interaction for visit rate; Table 4.1).

*Phaethornis striigularis* hummingbirds were more likely to make single- versus two-flower visits in response to some nectar treatments in *C. friedrichsthaliana* plants ($F_{5, 19.6} = 3.11, P = 0.031$; Figure 4.3). All three visit types were equally likely to occur in plants with either female-biased or unbiased nectar production. However, plants with male-biased nectar production were nearly twice as likely to receive a single-flower visit and half as likely to receive a female-then-male visit (linear contrasts for female-then-male visit: $t > 3.61, P < 0.0005$). In contrast to other visit types, visit types that could result in geitonogamous inbreeding (male phase then female phase visit) occurred at a constant rate across treatments, at about 32-34% of visits (linear contrasts for male-then-female visit: $t < 1.4, P > 0.16$, Figure 4.3).
Figure 4.3 Types of visits made by *Phaethornis striigularis* to two-flowered *Chrysothemis friedrichsthaliana* plants with male-biased, female-biased, and unbiased nectar production. Percentages based on total numbers of visits to 33 individual plants, each observed three times in the same experiment as Figure 4.2. Six hundred fifty-three visits were made by at least 11 different hummingbirds over the three flowering seasons of study. The proportions of male-to-female visits, which can cause geitonogamous inbreeding, were similar across treatments; See Results for statistical tests.

**Pollinator Responses to Nectar in Artificial Flowers**

The probability of an artificial flower receiving a *P. striigularis* visit was strongly dependent on the flower’s nectar volume ($F_{3,840.5} = 77.22$, $P < 0.0001$). It was not, however, affected by the volume of its companion flower ($F_{3,736.4} = 0.23$, $P = 0.88$) nor by their interaction ($F_{3,871.5} = 0.87$, $P = 0.46$; Figure 4.4). Flowers with 2, 3, 4 or 6 µL all had high (at least 93%) and statistically equivalent probabilities of being visited, whereas flowers with 0 µL all had similarly low (less than 25%) probabilities of being visited. Compared with nectar volume, the overall nectar treatment was relatively unimportant in predicting pollinator response, and the only important difference among volumes was between 0 and greater than 0 µL.
*Phaethornis striigularis* hummingbirds were more likely to make single- versus two-flower visits to plants with certain patterns of nectar variability within artificial flowers. Specifically, plants with one nectarless artificial flower received many more single-flower visits than plants with two rewarding flowers (overall test: $F_{8,698} = 29.97, P < 0.0001$; linear contrasts for all plants with two rewarding flowers vs. all plants with one nectarless flower: $t_{1,698} > 9.2, P < 0.0001$; Figure 4.5). By offering at least some nectar in both flowers, plants more than doubled their chances of receiving a high-to-low visit and more than quadrupled their chances of receiving a low-to-high visit. There were no differences, however, in visit types among plants with two rewarding flowers (4-2 and 6-2), nor among plants with one unrewarding flower (2-0, 4-0, and 6-0; all linear contrasts: $t < 1.1, P > 0.31$).

![Figure 4.4](image)

**Figure 4.4** Probabilities of *Phaethornis striigularis* visits to artificial flowers on *Chrysothemis friedrichsthaliana* plants with six different nectar production patterns. Treatment names correspond with the volumes added to each plant, one bar per artificial flower. Five different hummingbirds visited the array containing treatments 2-0, 4-0, 4-2, and 6-2 (32, 32, 32, 32 foraging bouts per bird per treatment, respectively), and two hummingbirds visited the array with 6-0, 4-2 and 3-3 (24, 24, 48). Least square means with different lowercase letters are significantly different (Tukey-Kramer adjusted, $\alpha = 0.05$), and error bars represent $\pm 1$ SE.
Figure 4.5 Types of visits made by *Phaethornis striigularis* to artificial flowers on *Chrysothemis friedrichsthaliana* plants, in response to six different nectar production patterns. Raw percentages for artificial flowers are based on foraging trials using two to five different hummingbirds, depending on the treatment. Plants offering some nectar in both flowers received more low-to-high and high-to-low moves, relative to plants with one flower unrewarding. Plants in treatment 3-3 (not shown) received the same proportions of single and two-flower visits as 4-2 and 6-2; see Results for statistical tests.

Plants in treatment 3-3 were similar to other plants with two rewarding flowers and different from those with one rewarding flower. They had a less than 20% probability of receiving a single-flower visit, as was observed in plants with two rewarding flowers (linear contrast against 4-2: \( t_{1,755} = 0.87 \ P = 0.39 \)), but not in plants with only one rewarding flower (linear contrast against 6-0: \( t_{1,755} = 5.06 \ P < 0.0001 \)).

All five hummingbirds in the artificial flower experiment used similar strategies when faced with within-plant variability in nectar. Visit probabilities and visit types were similar across hummingbirds, independent of nectar treatment (per-flower visit probability: \( F_{4,162.1} = 0.05, \ P = 0.99 \); per-plant visit types: \( F < 1.813, \ P > 0.36 \)). Hummingbirds also responded similarly to specific nectar volumes within flowers; they all tended to avoid flowers with no
nectar and tended to visit flowers with 2, 3, 4 or 6 µL at equal frequencies (hummingbird ×
treatment for per-flower visit probability: $F_{14,1200} = 1.21, P = 0.33$).

**Costs of Inbreeding**

Seed production and seed quality in *C. friedrichsthaliana* did not change in response to
pollination with inbred versus outcrossed pollen (Table 4.2; Figure 4.6). Number, mass,
germinability, and viability of seeds were equivalent between hand-pollination treatments,
averaged across 2004 and 2005 (Table 4.2). There was a trend for outcrossed seeds to be slightly
more germinable and viable than inbred seeds (Figure 4.6), but in no cases were differences
statistically significant (Table 4.2). There were large differences between experiments
performed in 2004 and 2005 for all response variables (Table 4.2). Averaged across all
treatments, flowers produced $1154 \pm 43$ seeds (LS Means ± SE) in 2004 and $980 \pm 48$ seeds in
2005. Germination and viability were high in 2004, at $65 \pm 5$ % and $70 \pm 7$ % of seeds,
respectively, whereas in 2005, only $19 \pm 4$ % of seeds germinated and $25 \pm 5$ % were viable.

**Table 4.2** Results of 2-way ANOVAs on seed production and seed quality of *Chrysothemis
friedrichsthaliana* in response to hand pollination treatments (see also Figure 4.6). Treatments
were applied with a *Phaethornis* sp. bill and consisted of pollination by two virgin male-phase
flowers from the same plant (inbred) or two virgin male-phase flowers on a different plant
(outcrossed). Experiments were replicated in the flowering seasons of 2004 and 2005.
Germination trials were performed ~5 months after seed collection, and each lasted 23 d. Plant
was a random effect in all models. Numerator degrees of freedom=1 for all effects and
variables; ddf=denominator degrees of freedom.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Seed count (no./flower) ddf</th>
<th>Mean weight per seed (mg) ddf</th>
<th>Percent germination ddf</th>
<th>Percent viable ddf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>58 0.01 0.93</td>
<td>40 0.06 0.80</td>
<td>32 3.27 0.08</td>
<td>32 1.05 0.31</td>
</tr>
<tr>
<td>Year</td>
<td>34 7.28 0.01</td>
<td>36 8.10 0.007</td>
<td>37 32.54 &lt;0.0001</td>
<td>37 31.21 &lt;0.0001</td>
</tr>
<tr>
<td>Year × Treatment</td>
<td>58 0.55 0.46</td>
<td>20 2.38 0.13</td>
<td>32 0.03 0.86</td>
<td>32 0.01 0.93</td>
</tr>
</tbody>
</table>
Figure 4.6 Seed production and quality in *Chrysothemis friedrichsthaliana* following hand-pollination with inbred and outcrossed pollen in 2004 and 2005. Costs of inbreeding are measured in terms of: (A) number of seeds produced per capsule, (B) percentage of seeds that germinated, (C) the mean seed weight per seed, and (D) percentage of seeds that were viable. Germination trials were performed on 100 seeds each, and lasted 23 d. Least square means with different lowercase letters are significantly different (Tukey-Kramer adjusted, $\alpha = 0.05$), and error bars represent ± 1 SE.

**DISCUSSION**

Experimental results suggest that within-plant nectar variability potentially has two adaptive functions in *Chrysothemis friedrichsthaliana*: to promote male-phase visits and to reduce inbreeding. Hummingbird pollinator *Phaethornis striigularis* generally preferred flowers that contained more nectar, which supports the remaining prediction of the sexual selection hypothesis. Variable rewards in *C. friedrichsthaliana* may also reduce maladaptive inbreeding,
since *P. striigularis* made more single-flower visits to plants with certain patterns of within-plant nectar variability. Even so, the responses of *P. striigularis* and *C. friedrichsthaliana* align with only a subset of focal predictions of the sexual selection and inbreeding avoidance hypotheses. Below, I assess how well current hypotheses explain male-biased nectar production in *C. friedrichsthaliana*, and when appropriate, I discuss their potential relevancy to other patterns of within-plant variability in nectar.

**Sexual Selection Hypotheses**

*Phaethornis striigularis* hummingbirds were able to detect and respond to varying nectar volumes in real and artificial flowers, suggesting that hummingbird-mediated sexual selection is possible in *C. friedrichsthaliana*. Foraging observations at unmanipulated *C. friedrichsthaliana* flowers corroborate these findings, since *P. striigularis* both preferred male-phase flowers and responded to nectar variability within flowers during the day (Carlson, 2007). The ability to track nectar within flowers is not unique to *P. striigularis*; many species of hummingbirds increase visit rates to real or artificial flowers with higher rewards, either when visual cues are present (Zimmerman, 1983; Gass and Sutherland, 1985, Miller et al., 1985; Melendez-Ackerman et al., 1997) or absent (Miller et al., 1985; Hurly, 1996; Irwin, 2000; Healy and Hurly, 2001). Preferences for more-rewarding flowers are also observed in other pollinator taxa, such as bumble bees and honey bees (e.g., Aizen and Basilio, 1998; Bell et al., 1984; Gonzalez et al., 1995; Johnson and Nilsson, 1999). This study is one of the few to demonstrate that nectar directly contributes to hummingbird preferences for nectar in real flowers, and it provides further evidence that sexual selection may maintain male-biased nectar production in this species.

Despite general agreement with sexual selection predictions, *P. striigularis* responses to nectar manipulations were nevertheless often context dependent. Hummingbirds altered visit rates to real flowers in response to nectar, but their behaviors were also influenced by floral
gender independent of nectar production. In plants with artificial flowers, nectar had a significant effect on behavior, but flowers containing two or more \( \mu \)L nectar were all visited at equivalent rates. Below, I consider possible explanations for these two unexpected results, and I discuss their implications for the sexual selection hypothesis.

**Context Dependency in Real Flowers**

The sexual selection hypothesis predicts that nectar is the primary cause of increased visits to male-phase flowers. Experimental results show, however, that both nectar and floral gender affect *P. striigularis* behavior. *Phaethornis striigularis* increased visits with nectar production in female-phase flowers, but they visited all male-phase flowers at high and constant rates. I interpret these behaviors as evidence that *P. striigularis* has innate or learned preferences for the male-phase phenotype in addition to preferences for nectar, since they always avoided unrewarding flowers when gender cues were absent (i.e., in artificial flowers). Nevertheless, morphological differences between male- and female-phase flowers are slight, potentially limited to the relative positions of anthers and stigmas. Given such small differences, morphology is unlikely to be the underlying cause of *P. striigularis* preferences for male-phase *C. friedrichsthaliana* flowers. I expect that male-phase flowers are revisited persistently not because they are easier to feed from or encounter (e.g., Waser and Price, 1981; 1985; Temeles et al., 2002), but rather because hummingbirds anticipate more nectar in male- than female-phase flowers.

*Phaethornis striigularis* hummingbirds may expect male-phase flowers to be more rewarding based on their experience in the population at large. Individual hummingbirds visit single, unmanipulated *C. friedrichsthaliana* plants up to 27 times daily (Carlson, 2007) and each plant is likely one of hundreds visited on each foraging bout (J. E. Carlson, personal observation). Thus, over a single flowering season, each hummingbird has tens of thousands of
opportunities to learn that male-phase morphology confers higher nectar production, and they may benefit by doing so. By making decisions based on gender specific cues, hummingbirds should save time by avoiding persistently less-rewarding flowers while remaining faithful to temporarily empty male-phase flowers (e.g., when recently drained by a competing hummingbird). I therefore expect that a lifetime’s worth of exposure to male-biased nectar production trains *P. striigularis* to rely heavily on visual cues in *C. friedrichsthaliana*. For the same reasons, hummingbirds may also occasionally disregard information gleaned from current or recent visits, thus explaining their preferences for male-phase flowers even when rewards were low.

Hummingbird pollinators prefer visual cues over nectar volumes in other plant taxa. Melendez-Ackerman and Campbell (1998) experimentally disassociated color cues from nectar and morphology in *Ipomopsis*. At their study site, red-flowered *I. aggregata* grew near a white-flowered congener and their pink-flowered hybrid, and the latter two taxa produced significantly less nectar than *I. aggregata*. They found that real flowers painted red were preferentially visited over real flowers painted pink or white, regardless of nectar content and underlying morphology. The authors suggested, as I do, that such findings are best interpreted within the context of lifetime experience and preferences formed at larger spatial scales. In an earlier study, Melendez-Ackerman and others (1997) found that when the same hummingbird species was exposed to altered rewards at a larger scale, they did switch preferences from red to white flowers when white flowers were more rewarding. This suggests that if hummingbirds encounter plants with atypical patterns frequently enough, they may learn to ignore misleading visual cues and subsequently rely on new visual or spatial cues (e.g. Healy and Hurly, 2001). Thus, with sufficient exposure, *P. striigularis* could eventually increase visit rates to female-phase flowers in *C. friedrichsthaliana* plants with female-biased or unbiased rewards.
Context-Dependency in Artificial Flowers

The sexual selection hypothesis predicted that *P. striigularis* should make more visits to artificial flowers with increasing nectar volumes, but they only discriminated between 0 and ≥2 μL nectar. Optimal foraging may be used to explain these behaviors. Theory predicts that hummingbirds should not pass up any reliable source of nectar in a patch (or multi-flowered plant) if all flowers are above a profitability threshold, possibly because other patches are of lesser value; or if little energy is spent moving within versus between patches (Stephen and Krebs, 1986). Both of these predictions are likely to be true for *P. striigularis*. First, experimentally-manipulated real and artificial flowers often contained more nectar than flowers on neighboring plants (Carlson, 2007), which may have rendered all experimental flowers especially attractive to *P. striigularis*. Second, the amount of energy required to move within a plant or patch was likely relatively small. Given these observations, I expect that nectar volumes added to artificial flowers were simply too large to have elicited fine-scale discrimination by hummingbirds. It is possible, however, that even under natural conditions, *P. striigularis* discriminates strongly against flowers on visited plants only if they are entirely or very close to unrewarding. Unrewarding *C. friedrichsthaliana* flowers do occur naturally; female-phase flowers are often nectarless in the mid and late afternoon when discrimination against the female-phase is the strongest (Carlson, 2007).

If the context-specific responses described above are representative of *P. striigularis* behavior on larger temporal and spatial scales, pollinator-mediated sexual selection may still favor male-biased nectar production, but in fairly specific ways. Selection is unlikely to act directly on male-phase production, since hummingbirds visit more- and less-rewarding male-phase flowers with equal frequencies. *Phaethornis striigularis* does respond to increased nectar in female-phase flowers, however, suggesting that if increased visits were positively associated
with fitness (e.g., if pollen limitation was strong; Ashman et al., 2004; Knight et al., 2005),
selection could act directly on production during that phase. Nevertheless, Carlson (2007)
showed that female fecundity in *C. friedrichsthaliana* requires one or fewer visits to maximize
seed set, such that plant fitness is unlikely to increase with additional female-phase visits. As a
result, plants that produce as much or more nectar in female- relative to male-phase flowers
would likely suffer the energetic costs of nectar production (e.g., Southwick, 1984; Pyke, 1991;
but see Ordano and Ornelas, 2005) with no fitness benefits. Thus, male-biased nectar production
may currently be maintained by negative or neutral selection on female-phase nectar production
rather than by positive selection on male-phase nectar production.

**Inbreeding Avoidance Hypotheses**

**Pollinator Responses to Nectar in Real and Artificial Flowers**

*Phaethornis striigularis* behaviors at nectar-manipulated plants provide limited evidence
for the inbreeding avoidance hypothesis. In the absence of gender cues, *P. striigularis* made
more single-flower visits in response to some variable-reward treatments. This would likely
result in reduced geitonogamy in plants with male-biased as opposed to unbiased nectar
production. Nevertheless, the same behaviors in plants with female-biased nectar production
would even further reduce geitonogamy, because low to high, geitonogamy-causing visits would
be drastically reduced. In sum, *P. striigularis* responses to variability could contribute to the
maintenance of male-biased rewards if costs of geitonogamy were high, but would require that
another selection pressure, such as sexual selection, originally directed the bias towards the
male-phase.

Even when inbreeding avoidance predictions were partially supported, the responses
were context dependent at both real and artificial flowers. Three predictions were not well met,
due to context-dependent responses. First, I predicted that plants should receive more single-
flower visits when rewards were variable between pairs of real flowers. Hummingbirds nevertheless responded identically to the female-biased (variable rewards) and unbiased treatments (constant rewards). Second, I predicted that plants should receive more single-flower visits when rewards were variable between pairs of artificial flowers. Experimental results showed that treatments including one empty flower received many single-flower visits, but those including two differentially-rewarding flowers received relatively few. Finally, I predicted that when hummingbirds visit both flowers on a plant with artificial flowers, they should most often move from high to low rewards. When approaching plants with at least 2 μL per flower, *P. striigularis* did not have a preferred visit order. When one flower was rewardless, however, two-flower visits most often started with the rewarding flower. Of these three context-dependent responses, the first two have been discussed under the sexual selection hypothesis. The third, however, warrants additional interpretation in the context of inbreeding avoidance.

The inbreeding avoidance hypothesis for gender-biased nectar production predicts that pollinators should visit flowers in order of decreasing rewards until encountering a flower below a profitability threshold (Pyke, 1978; Stephens and Krebs, 1986; Best and Bierzychudek, 1982; Carlson and Harms, 2006). This prediction seems most relevant for pollinators foraging within many-flowered displays with nectar gradients, but nevertheless applies when display sizes are small and unstructured, as in *C. friedrichsthaliana*. *Phaethornis striigularis* often moved from high to low rewards when biases were interpreted as strong (i.e., one flower was empty). Another study has also observed hummingbirds moving from rewarding to rewardless artificial flowers in small clusters (Hurly, 1996). These behaviors could be interpreted as beneficial for plants with variable rewards without gender-biased nectar production; otherwise, they would only reduce inbreeding if rewards were female-biased. Moves from high to low rewards within plants reduce geitonogamy and promote outcrossing in plants with female-biased nectar
production, but do the opposite in plants with male-biased nectar production (Carlson and Harms, 2006).

Phaethornis striigularis responses to strong within-plant variability meet a basic precept of the inbreeding avoidance hypothesis: that a lower proportion of visits are geitonogamous when rewards are variable. Nevertheless, a slightly different set of outcomes was predicted by the declining rewards hypothesis, which applies specifically to discriminating pollinators like P. striigularis. Carlson and Harms (2006) postulated that male-to-female moves should be most frequent within plants with male-biased rewards, and plants with unbiased rewards should be intermediate. The results presented here suggest that the latter part of this assumption was incorrect – both forms of within-plant variability should reduce geitonogamy more effectively than unbiased rewards, and female-biased rewards should reduce geitonogamy the most. Earlier predictions failed to recognize that single-flower visits could become more frequent as gender-biases became stronger. In this way, visit order should matter less than the frequency of single-flower visits for predicting geitonogamy.

Costs of Inbreeding

Current and previous studies confirm that C. friedrichsthaliana exhibits few immediate costs of self-fertilization. The following observations support this conclusion: maximal autogamy produces nearly as many seeds as a single outcrossed visit (Carlson, 2007); and two geitonogamous visits are equivalent to two outcrossed visits in terms of seed quality and quantity (current study). Even if statistical power were increased by larger sample sizes, non-significant trends suggest that outcrossing could only improve germination by 11%. These findings therefore provide very limited support for the inbreeding avoidance hypothesis in this species. It is possible, however, that the greatest costs of inbreeding in C. friedrichsthaliana emerge as seedlings approach adulthood. For example, seedlings produced through inbreeding may grow
less quickly or set fewer or smaller seeds, relative to those produced through outcrossing (e.g., Wolfe, 1993). If, however, inbred and outcrossed offspring remain equally fit as they mature and reproduce, selection may actually favor inbreeding, rather than reduce it (e.g., Morgan et al., 1997; Fishman and Wyatt, 1999).

Estimates of seed production were fairly consistent between pollination treatments within years, but yearly estimates were notably dissimilar. Seed production and quality were roughly three times higher in the group of plants studied in 2004, relative to that of 2005. This is perhaps because climatic conditions differed between years (J. E. Carlson, unpublished data).

Furthermore, both the 2004 and 2005 inbreeding experiments provided higher overall estimates of seed production than previously reported for this species. This may be because the previous study used considerably smaller plants for fecundity estimates (Carlson, 2007). A positive relationship between plant size and seed production has been observed in many species (e.g., Klinkhamer and de Jong, 1987; Dudash, 1991; Lawrence, 1993; Stöcklin and Favre, 2004), but remains to be tested directly in *C. friedrichsthaliana*. If larger plants are more fecund, they may also be more capable of overriding costs of inbreeding than small plants (Wolfe, 1993). Such maternal effects are pervasive in plants, and they should be particularly strong during the early life stages of offspring (Roach and Wulff, 1987; Wolfe, 1993). Given this possibility, the low costs of inbreeding I observed in large, vigorous plants may not be representative of the population as a whole.

Despite weak evidence that there exist costs of inbreeding in *C. friedrichsthaliana*, one important cost specific to geitonogamous inbreeding remains unassessed. This cost is pollen discounting, or the wastage or loss of pollen that could have fertilized outcrossed ovules (de Jong et al., 1993; Fishman, 2000). High levels of geitonogamy may reduce the probability that pollinators deliver pollen to receptive stigmas on other plants, as opposed to already-saturated
stigmas on the same plant. Pollen discounting may thereby impose reproductive costs even when inbreeding does not, selecting for individuals that reduce geitonogamy (e.g., by presenting variable rewards within plants). In *C. friedrichsthaliana*, the degree of pollen discounting is unknown, although it undoubtedly occurs, given that 30% of visits are from male-to-female flowers within a plant. Only a few studies have experimentally assessed pollen discounting (e.g., Fishman, 2000), and further research is needed to clarify its importance to the inbreeding avoidance hypothesis.

**Summary**

Experiments using *P. striigularis* and *C. friedrichsthaliana* provide moderately strong support for the sexual selection hypothesis for male-biased nectar production and weaker support for the inbreeding avoidance hypothesis. In combination with earlier observations that pollinator visits are more limiting to male than female fecundity in *C. friedrichsthaliana* (Carlson 2007), these findings are the strongest evidence for sexual selection on nectar production thus far. Some predictions of the sexual selection hypothesis have been matched in other dichogamous species with male-biased nectar production (e.g., Bell et al., 1984; Devlin and Stephenson, 1985; Aizen and Basilio, 1998). Support for the inbreeding avoidance hypothesis is more limited, based predominately on a few species with female-biases and multiple-flower displays (e.g., Pyke, 1978; Best and Bierzychudek, 1982). This research stands out among previous studies because it derives and simultaneously tests multiple predictions of natural and sexual selection theory using both field observations and experimental manipulations. By completing the links between variable nectar rewards, pollinator response, and plant fitness in *C. friedrichsthaliana*, I provide a strong mechanistic basis for the adaptive benefits of variable rewards caused by male-biased nectar production.
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Chapter 5

The Benefits of Bathing Buds: Water Calyces Protect Flowers from a Microlepidopteran Herbivore

INTRODUCTION

Herbivory on plant reproductive tissues can have severe consequences for plant fitness, thereby promoting the evolution of defensive floral traits. Floral traits that deter herbivores include physical defenses, such as resins or sharp trichomes (Armbruster, 1997; Abdala-Roberts and Parra-Tabla, 2005), extrafloral nectaries that attract enemies of herbivores (e.g., Horvitz and Schemske, 1984; Sugiura et al., 2006), or enlarged sepals or bracts (e.g., Armbruster and Mziray, 1984; Delph et al., 1996). Flowers may also be defended by constitutive or induced chemicals, such as glucosinolates or nicotine (McCall and Irwin, 2006). Although recent work experimentally demonstrates the role of chemicals in floral defense (e.g., Strauss et al., 2004; McCall and Karban, 2006), physical defenses have received considerably less attention.

The outermost floral whorl– composed of sepals (or a calyx when the sepals are fused)–is generally thought to function to protect reproductive tissues (Delph et al., 1996; Endress, 1996; Armbruster, 1997). Although floral herbivores are presumed to be repelled by various sepal modifications (e.g., McCall and Irwin, 2006; Sugiura et al., 2006), such claims are rarely supported experimentally (but see Armbruster and Mziray, 1984). We tested the adaptive function of an unusual sepal phenotype: the water calyx.

Water calyces hold and often secrete liquid, causing buds to develop under an aqueous or mucilaginous mixture (Burtt and Woods, 1975; Endress, 1996; Figure 5.1A). Water calyces were first described in Spathodea campanulata (Bignoniaceae) over 100 years ago (Treub, 1889).
and have since been documented in over ten species of at least six plant families (Endress, 1996; J. E. Carlson and K. E. Harms, unpublished data), suggesting multiple evolutionary origins of the trait. Water calyx morphology is also variable among families: sepals may be partially or completely fused throughout bud development, and liquid-secreting trichomes may be present or absent on adaxial surfaces.
Skutch (1992) hypothesized that water calyces dissuade herbivores. A comparable function is provided by liquid-filled bracts of some *Heliconia* species, which reduce herbivore damage to floral parts (Wootton and Sun, 1990). A non-exclusive alternative is that calyx liquid protects buds from desiccation during drought (Burtt and Woods, 1975), or may otherwise be important to water-related physiology of flowers. We tested these functional hypotheses in the neotropical plant *Chrysothemis friedrichsthaliana* (Hanst.) H.E. Moore (Gesneriaceae; Figure 5.1A, B) by comparing rates of herbivory and bud abortion in calyces that had been drained of liquid, relative to unmanipulated water calyces.

**MATERIALS AND METHODS**

We monitored over 350 *C. friedrichsthaliana* plants from a large population on the Osa Peninsula of Costa Rica, from August to October within the flowering seasons of 2005 and 2006 (see Carlson, 2007 for description of study species and site). Plants were routinely surveyed to identify the most important floral herbivores and their associated damage. We confirmed preliminary observations by dissecting hundreds of flowers, rearing larvae, and viewing over 2500 daylight hours of video on unmanipulated *C. friedrichsthaliana* flowers (collected for a pollination study; Carlson, 2007).

The cup-like calyces of *C. friedrichsthaliana* (Figure 5.1A) open 2-3 weeks before the corollas and are lined with liquid-secreting trichomes. After the 2-d protandrous flowers open and abscise, calyces persist for an additional 4-5 weeks (J. E. Carlson, unpublished data). We chose buds in an early developmental stage (i.e., in unopened calyces) and randomly assigned at least 80 buds to each of three treatments: calyx unmanipulated, calyx drained, and calyx drained then refilled. Refilled calyces received liquid from the same or neighboring calyces, to maintain the same chemical composition as unmanipulated controls.
Treatments were applied each afternoon for 21 days, or until the flower was consumed or reached anthesis (i.e., flower opened). Number of days until anthesis did not vary among treatments (J. E. Carlson, unpublished data). After one season, we found that neither floral herbivory nor bud abortion differed between unmanipulated calyces and calyces drained then refilled (linear contrasts for both variables: $t < 0.13, P > 0.90$), suggesting that handling caused no additional bud loss. Thus, we collapsed these two treatments into a single ‘calyx undrained’ treatment, and we pooled both years’ data.

Each day that treatments were applied, we recorded the condition of individual buds. All buds experienced one of three fates by the experiment’s end: lost to herbivores, lost to premature abortion (remaining small and undeveloped), or open and intact. We subdivided herbivore losses into two categories: flowers were consumed completely prior to opening, or flowers were sterilized prior to opening, i.e., anthers and stigmas were consumed entirely, but corollas opened at anthesis.

To determine if buds in water-filled calyces were less susceptible to sterilization, complete consumption, or to premature abortion (possibly due to desiccation), we performed three binary logistic regressions comparing drained and undrained calyces. First, we compared the probability of flower sterilization between treatments, only using flowers that reached anthesis. Second, we compared the probability of complete flower consumption between treatments, using all flowers. Third, we compared the probability of bud abortion, again using the total number of flowers. We coded all response variables as binary on a flower and tested each using the Glimmix procedure in SAS 9.1, including year as a random effect.

RESULTS

A moth in the family Alucitidae (*Alucita* sp. cf. *flavicincta* Walsingham; Figure 5.1C) was the most detrimental floral herbivore of *C. friedrichsthaliana*, destroying 38% of
unmanipulated flowers in 2005 and 19% in 2006. Adult moths oviposit in immature *C. friedrichsthaliana* buds, and larvae remain inside the buds until anthesis, completely consuming the developing anthers and stigmas, but not the corolla. All flower sterilization could be attributed to the alucitid larvae, and oviposition scars on buds reliably indicated larval infestation, even at early stages in bud development.

Calyx draining more than doubled the odds that individual flowers would be larvae-infested and lack reproductive structures upon opening, relative to flowers in water-filled calyces ($F_{1, 241} = 6.87$, $P = 0.009$, odds ratio: 2.18, 95% CI: 1.21-3.92; Figure 5.2). Survival of alucitid larvae was high and invariable across treatments; more than 98% of buds with oviposition scars in both drained and undrained calyces were sterile and contained larvae upon opening. Therefore, differences in flower loss were most likely caused by reduced oviposition in immersed buds, rather than reduced larval survival.

Generalist herbivores, including grasshoppers and katydids, were responsible for some additional herbivory, consuming 4% of unmanipulated flowers. In contrast to alucitid moths, these herbivores consumed flowers completely and did not respond to calyx-draining treatments ($F_{1, 355} = 0.02$, $P = 0.88$; Figure 5.2). Finally, a quarter of buds were aborted at an early developmental stage, but abortion was not significantly more likely in drained calyces ($F_{1, 355} = 1.21$, $P = 0.27$; Figure 5.2). Almost all aborted buds lacked alucitid oviposition scars, suggesting that abortion generally occurred prior to alucitid attack, and that moths avoided buds that would be subsequently aborted. Thus, we inferred that bud abortion was unlikely to be caused by larval infestation.
**DISCUSSION**

Our experiments indicate that water calyces serve as a defense against floral herbivores. This result supports the wide-spread but inadequately-tested view that sepals are predominately protective in function (e.g., Delph et al., 1996; Endress, 1996). Although floral herbivores and other enemies are often recognized as important contributors to the evolution of floral phenotype (e.g., Linhart, 1991; Irwin et al., 2004; McCall and Irwin, 2006), our study is among the few that experimentally test evolutionary hypotheses for a physical floral defense.

In *C. friedrichsthaliana*, a liquid-filled calyx protects buds from ovipositing moths, but does not otherwise alter bud survival and development. Bud abortion was fairly high, but was invariant between filled (calyx undrained) and empty (calyx drained) conditions. This suggests that the water calyx is unlikely to serve an anti-desiccation or water balance-related function.
The causes of bud abortion were not determined in this study, although other studies have shown that low availability of plant resources can cause high bud abortion rates (e.g., Stöcklin, 1997).

Our results support Skutch’s (1992) original prediction that C. friedrichsthaliana’s water calyces function for herbivore defense. The defense is only partial, however, as generalist herbivores were unaffected by filled calyces. Even so, generalists consumed relatively few flowers overall. Flower loss was much greater to alucitid moths, which were the only herbivores deterred by bud submersion. The moths have a wingspan of ~11 mm, and they have short ovipositors. Thus, it is unlikely that they can reach buds through 5 to 20 mm of calyx liquid – the range of depths typically observed (J. E. Carlson, unpublished data).

Oviposition was significantly reduced in water-filled calyces, yet alucitid larvae still destroyed a quarter of buds in the undrained treatment. We explain this observation as follows: calyces occasionally dry out on sunny days, providing opportunities for ovipositing moths to reach buds. Undrained calyces were encountered empty on an average of 17% of days preceding floral anthesis (SD = 0.14, N = 24). Most empty calyces were full again within 24 hours, although filling rates were variable among plants (Skutch, 1992; J. E. Carlson, unpublished data).

Calyx phenotype may influence the behaviors of floral visitors other than herbivores; for example, pollinators are attracted to brightly colored sepals (or calyces) on some plants (e.g., Schemske et al., 1996). We doubt, however, that the water calyx of C. friedrichsthaliana is used by its hummingbird pollinator (Phaethornis striigularis) as an indicator of floral rewards, because the yellow-green calyces do not reliably indicate nectar availability. Nectar robbers or thieves may also selectively favor floral modifications (Galen, 1999; Irwin et al., 2004). Nevertheless, the non-pollinating, nectar-thieving ants that visit C. friedrichsthaliana are not deterred by water-filled calyces; even small ants can bypass the water calyx and enter the mouth.
of the corolla. Furthermore, flowers with ants do not receive fewer pollinator visits, and visiting ants generally do not damage reproductive parts (J. E. Carlson, unpublished data). Thus, alucitid moths appear to exert the single most important selection pressure currently acting on _C. friedrichsthaliana_’s water calyces.

Cup-like water calyces may function predominately for herbivore defense, yet individual floral traits do not evolve in isolation. For example, pollinators and nectar robbers may influence calyx phenotype through genetic correlations with other floral traits (e.g., Armbruster, 1997). Furthermore, selection for one trait, e.g., upright, water-filled calyces, may constrain selection for optimal performance of another, e.g., corolla orientation for most-effective pollination. Such complex interactions cannot be detected, however, until the effects of all visitors and trait pleiotropisms are known. Detailed studies may reveal how selection by floral herbivores interacts with selection by other visitors, to ultimately shape floral evolution.

**LITERATURE CITED**


Chapter 6
Conclusions

Traits of extant flowers are currently maintained by a multitude of selective agents, including both mutualists and antagonists. In this dissertation, I demonstrated how pollinator-mediated sexual selection likely maintains male-biased nectar production in *Chrysothemis friedrichsthaliana*. I also illustrated how the fitness-reducing effects of microlepidopteran moths may provide an adaptive function for cup-like, liquid-filled calyces. In the following sections, I review major findings, and I propose additional avenues of research motivated by these results.

**SUMMARY**

**Male-Biased Nectar Production**

This dissertation tested the sexual selection and inbreeding avoidance hypotheses for male-biased nectar production in a representative dichogamous plant (Carlson and Harms, 2006). My observations and experiments over three consecutive flowering seasons strongly suggested that male-biased nectar production in *C. friedrichsthaliana* is a sexually-selected trait, and was less likely to be maintained by natural selection favoring inbreeding avoidance (Carlson, 2007; Chapter 4). In accordance with two basic assumptions of sexual selection theory in plants, high quality pollinator visits were at least occasionally in short supply, and male fecundity (i.e., number of seeds sired) was more strongly limited by pollinator visits than was female fecundity (Carlson, 2007). Furthermore, nectar rewards were consistently higher during the male floral phase, despite genetically or maternally-based variability among plants as well as seasonal variation in rainfall. Hummingbird pollinator *Phaethornis striigularis* (Trochilidae: Phaethornithinae) more frequently visited male-phase flowers, spending 53% more time per visit and making 86% more visits to the male relative to the female phase (Carlson, 2007). Finally, *P.
*Phaethornis striigularis* responded directly to nectar manipulations in real and artificial flowers, although they tracked rewards more carefully in female-phase flowers and only discriminated against near-empty flowers (Chapter 4).

*Phaethornis striigularis*’s preferences for nectar in real and artificial flowers provided very limited support for the inbreeding avoidance hypothesis. *Phaethornis striigularis* made more single-flower visits and fewer moves from low to high rewards in response to certain variable-reward patterns in artificial flowers. The latter behavior would most drastically reduce self-pollination if rewards were female-biased. When visiting plants with real flowers, however, *P. striigularis* did not make more single-flower visits or female-to-male moves in response to variable as opposed to constant rewards. The inbreeding avoidance hypothesis was further refuted by the lack of maternal costs of self-fertilization, in terms of seed number, size, germinability or viability (Chapter 4).

This dissertation tested the two leading hypotheses for male-biased nectar production, i.e., those that had the strongest theoretical and empirical support. Other factors listed by Carlson and Harms (2006) were not directly examined (e.g., resource tradeoffs, floral enemies, and genetic correlations), but were unlikely to be particularly important in *C. friedrichsthaliana*. First, resource tradeoffs are unlikely to constrain selection for unequal nectar production between phases, although they may prevent plants from producing more nectar overall (see also Carlson and Harms, 2006). Second, pollen robbing stingless bees and nectar robbing ants do not preferentially visit male-phase *C. friedrichsthaliana* flowers in response to nectar. Thus, the selection pressures of floral enemies are unlikely to act directly upon nectar production patterns.

Genetic correlations among floral traits in *C. friedrichsthaliana* were not assessed in this dissertation. Though genetic correlations may exist, selection on a fitness-enhancing trait like nectar production should overwhelm selection on most other pleiotropically-linked floral traits.
Nectar is related to fitness in *C. friedrichsthaliana* through the following relationships. Pollinator visits were longer and more frequent to flowers with nectar (Chapter 4), and flowers that received more visits had higher fecundity, predominately because they sired more seeds (Carlson, 2007). Together, these relationships reveal the mechanistic basis for the adaptive function of male-biased nectar production, which strongly suggests that male-biased rewards did not evolve through selection on a pleiotropically-linked trait. By field testing theoretical predictions and completing links between nectar, pollinator behavior, and plant fecundity, this dissertation provides the most comprehensive support for sexual selection on nectar thus far.

**Water Calyces**

In Chapter 5, I demonstrated that the water calyx of *C. friedrichsthaliana* reduced floral herbivory by submerging buds during their development (Carlson and Harms, 2007). The most detrimental floral herbivores in the study population, larvae of an alucitid moth, was partially deterred when water calyces functioned properly (i.e., when they were filled with liquid, as opposed to experimentally drained). Variation in filling rate among plants indicates that this trait could respond to selection by floral herbivores. It is currently unclear, however, how much of observed variability is due to environmental versus genotypic effects.

This study is the first to show that an alucitid moth, whose larvae are often bud-boring, may act as a selective agent on floral morphology. The family Alucitid has a cosmopolitan distribution (Scoble, 1992), but the small, fragile moths have very rarely been studied. There are no published studies describing the biology of any of the 24 described species of the family Alucitidae in the neotropics (K. Tuck, personal communication). Thus, this dissertation is the first work containing data on the biology or ecology of any neotropical alucitid.
FUTURE DIRECTIONS

The theoretical predictions and unique traits highlighted in this dissertation offer many avenues for future research. Below, I describe three potential research directions motivated by my results, involving other members of the genus *Chrysothemis*, other species with male or female-biased nectar production, and flowers of *C. friedrichsthaliana* as an integrated unit.

**Nectar Production and Pollination in the Genus *Chrysothemis***

The genus *Chrysothemis* has ten members, distributed in lowland habitats of Nicaragua south to Brazil, as well as the West Indies (Skog, 1978; VAST, 2007). Basic biological and ecological data are available only for *C. friedrichsthaliana*, which currently precludes comparisons among species. Studies of congeners would complement present work, allowing for elegant comparisons of floral phenotypes and the selection pressures acting on each species. Among known congeners, *C. pulchella* would be of particular interest because its range overlaps with *C. friedrichsthaliana* in Panama (Skog, 1978). These two species are morphologically similar, although the water calyces of *C. pulchella* are red, rather than yellow-green.

*Phaethornis striigularis* may pollinate *C. pulchella*, as well as other *Chrysothemis* species, since the range of *P. striigularis* reaches into northern Colombia and northwestern Venezuela (although not the West Indies; Hinkelmann and Schuchmann, 1997). Studies of the pollinators, reproductive biology, and nectar production patterns of *C. pulchella* and other congeners should provide valuable insights into the evolution of male-biased nectar production within the genus. These data, once mapped onto a phylogeny of *Chrysothemis* and related species, could also be used to trace the origins of male-biased nectar production as an adaptive floral trait. Phylogenetic, comparative analyses would greatly complement current data, which are concerned primarily with the ecological processes contributing to the trait’s maintenance, rather than its origin.
Congeneric comparisons among *Chrysothemis* species may also be utilized in future evolutionary studies of water calyces. There is variation in calyx phenotype within the genus *Chrysothemis*; for example, the calyx of *C. pulchella* is red and notably less inflated (Skog, 1978). Although the important floral herbivores are not known for any other *Chrysothemis* species, all *Chrysothemis* populations undoubtedly suffer some reproductive losses to floral herbivores. The selective pressures imposed by different herbivore assemblages may explain some of the diversity in calyx morphology among *Chrysothemis* species.

**Gender-Biased Nectar Production (GBNP) in Other Dichogamous Species**

Carlson and Harms (2006) listed 41 species with gender-biased nectar production (GBNP). Among these, nectar was biased toward the male phase in 21 species and toward the female phase in 20 species (Table 2.1). At least five species with male-biased nectar production from Table 2.1 exhibited some characteristics congruent with the sexual selection hypothesis. Additional data on these species, including *Impatiens capensis* (Bell et al. 1984), *Alstromeria aurea* (Aizen and Basilio, 1998), and *Lobelia cardinalis* (Devlin and Stephenson, 1985) could be used to assess the generality of the sexual selection hypothesis. It also remains to be shown that ecological processes demonstrated in this dissertation are occurring in other *C. friedrichsthaliana* populations.

Female-biased nectar production and its apparent associations with bee pollination and inbreeding avoidance remain understudied. Observations suggest that female-biased nectar production within protandrous racemes directs bee movements from female- to male-phase flowers, which may reduce within-plant pollen movement (e.g., Pyke, 1978 for *Delphinium* spp.; Galen and Plowright, 1985 for *Epilobium angustifolium*; Best and Bierzchudek, 1982 for *Digitalis purpurea*; reviewed in Carlson and Harms, 2006). The hypothesized reduction in geitonogamy has yet to be demonstrated, however. If future investigation shows that bee
foraging behavior reduces geitonogamy in any of the aforementioned species, the declining rewards hypothesis of Carlson and Harms (2006) would be supported.

A few plant species with female-biased nectar production lack nectar gradients, resulting in a unique reward distribution that has received very little attention. Species that exhibit this pattern include *Lavandula stoechas* (Gonzalez et al., 1995) as well as other *Lavandula* species (Upson and Andrews, 2004), and *Campanula rotundifolia* (Cresswell and Robertson, 1994). Relevant data on these species are very limited, making it difficult to predict which hypothesis most likely explains their nectar production patterns. Of the hypotheses listed by Carlson and Harms (2006), the following three are potential explanations for this atypical pattern of female-biased nectar production: the declining rewards hypothesis, the unpredictable rewards hypothesis, and the female-function version of the sexual selection hypothesis. Given the range of possibilities, these poorly studied species have great potential to serve as uniquely informative examples among those listed on Table 2.1.

Nectar production is not always biased in the same direction within GBNP taxa, which presents opportunities for study. Some lineages, species, and populations include representatives with male-, female-, or un-biased nectar production (Carlson and Harms, 2006). Examples of taxa that are consistently female-biased include *Delphinium* spp. (Pyke, 1978; Waddington, 1981; Cruden et al., 1983), and possibly *Lavandula* spp. (Upson and Andrews, 2004). Examples of taxa that are not consistently male- or female-biased include *Scrophularia* spp. (Olivencia and Alcaraz, 1993), populations of *Lobelia cardinalis* (Cruden et al., 1983 vs. Devlin and Stephenson, 1985; see Langenberger and Davis, 2002), and individuals within a population of *Lobelia deckenii* (Burd, 1992). Future work on the evolution of GBNP should focus on the latter group, that is, on closely related taxa that exhibit genetic variation in direction of bias. Study of
these taxa may reveal which selection pressures promote male-versus female-biased nectar production, without being confounded by large phylogenetic distances.

**The Integrated Flower of Chrysothemis friedrichsthaliana**

The floral traits examined in this dissertation may function predominately for male-biased pollinator attraction and herbivore defense, yet no trait evolves in isolation. Multiple floral visitors may impose selection on the same floral traits, potentially in the opposite directions or at differential strengths (e.g., Linhart, 1991; Brody, 1997; Galen, 1999; Strauss and Irwin, 2004). Such complex interactions have not yet been assessed in *C. friedrichsthaliana*, primarily because the effects of some visitors are unknown. Detailed studies of other visitors, including the Meliponine bee *Trigona fulviventris* and the assemblage of nectar-thieving ants, may reveal how selection by pollinators or floral herbivores interacts with selection by these visitors. The results presented here nevertheless capture the main effects of key mutualists and enemies of *C. friedrichsthaliana*. By describing how important visitors respond to traits and affect reproduction, this dissertation takes great strides towards a more complete understanding of how multiple interactors have shaped and maintain flowers.

**LITERATURE CITED**


Appendix A

Experimental Design Figures

RELATIONSHIPS BETWEEN POLLINATOR VISITS AND MALE VERSUS FEMALE FECUNDITY (CHAPTER 3)

**Figure A.1** Experimental design for testing the effects of cross-pollination success on male versus female fecundity. Male and female symbols represent real male- and female-phase flowers on *Chrysothemis friedrichsthaliana* plants growing at Centro Tropical of the Fundación Neotrópica in the Golfo Dulce Forestal Reserve on Osa Peninsula, Puntarenas Province, Costa Rica. Prior to experimental pollination, all flowers were unvisited (virgin). Pollen was transferred using a *Phaethornis* sp. bill, cleaned with ethanol following each use. Female-phase flowers were bagged following hand-pollination, and seed capsules were collected ~35 days later. Male fecundity was estimated as the total number of seeds sired by each male-phase flower (seeds set by female-phase flowers on nonfocal plants), and female fecundity was estimated as the number of seeds set by each focal female-phase flower. Fixed effects in the statistical model were cross-pollination treatment, floral gender and their interaction. Male- and female-phase flowers from the same plant were paired in analyses and assigned separate variances structures. Statistical analyses were performed using the Mixed procedure in SAS 9.1.3 (Cary, North Carolina, USA).
POLLINATOR RESPONSES TO NECTAR IN REAL FLOWERS
(CHAPTER 4)

Figure A.2 Experimental design for testing the responses of hummingbird pollinator Phaethornis striigularis to male-biased, female-biased, and unbiased nectar production. Nectar was manipulated in real male- and female- phase flowers (represented in Figure by male and female symbols) on Chrysothemis friedrichsthaliana plants. Nectar treatments were applied prior to and twice during a 2 h video-observation period, beginning at 0530 hours. Pollinator responses were measured as the number and duration of visits to male- and female-phase flowers, as well as the types of visits made to plants (moved from male- to female-phase flower within a plant, from female- to male-phase flower within a plant, or only one flower visited). Flowers from the same plant were considered split-plots, which accounted for their lack of independence in statistical analyses. Fixed effects were patch of plants (each approximately representing a single hummingbird), nectar treatment, floral gender and all interactions. Random effects were week, plant and year, the latter of which was modeled with heterogeneous variance. Statistical analyses were performed with the Glimmix procedure in SAS 9.1.3.
Figure A.3 Experimental design for testing the responses of hummingbird pollinator *Phaethornis striigularis* to within-plant nectar variability in artificial flowers. Four unique treatments were applied to the eight plants, in the amounts listed above the flowers. Nectar was added to artificial flowers attached to real *Chrysothemis friedrichsthaliana* plants on a low table within a natural patch of plants. Nectar treatments were applied once per hour from 1400-1600 hours, and one foraging bout was observed per hour. The experimental set-up for a second artificial flower experiment (with six nectar treatments) was similar, except that two plants were removed from the array, and only two hummingbirds completed the 6-d trial. Both experiments were combined in statistical analyses. Pollinator responses were measured as a binary variable, success or failure to visit a flower, and as multinomial variable, success or failure to make each of three visit types. For the binary analysis, there were five fixed effects: nectar volume of a flower, nectar volume of the companion flower, their interaction, hummingbird, and the interaction between hummingbird and a flower’s nectar volume. For the multinomial analysis, fixed effects were nectar treatment of the plant and hummingbird. Random effects were plant, day and trial. Flowers on the same plant were paired in statistical analyses to account for their lack of independence. Statistical analyses were performed using the Glimmix procedure in SAS 9.1.3.
Appendix B

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Editor, The Botanical Review
New York Botanical Garden
200th St. & Southern Blvd.
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Telephone: 718-817-8632
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To: Jane Carlson <jcarls3@lsu.edu>

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Vita

Jane Elizabeth Carlson was born on November 5, 1979, in Columbia, Missouri, to Carolyn A. and Douglas M. Carlson. She lived in Baton Rouge, Louisiana, when she was one year old, and she later moved to New York State, attending high school at Copenhagen Central School. Her outdoor education began in New York State, starting in the Catskills, and later migrating to the Tug Hill Plateau and the Finger Lakes regions, guided by two excellent natural historians, her parents. Jane’s father is a fisheries biologist for the New York State Department of Environmental Conservation, and her mother is a teacher of fourth grade and science enrichment. In 1998, Jane enrolled in Cornell University, where she had her first research experience in plant ecology, ornithology, and field biology. Jane also informally studied botanical and wildlife illustration in the Cornell Conservatory and Arboretum, which sparked her interest in the ‘why’ of floral traits. Jane completed an honor’s thesis on seed dispersal by gopher tortoises at Archbold Biological Station in Lake Placid, Florida, co-advised by Dr. Eric Menges and Dr. Peter Marks. Jane graduated from the Natural Resources Department at Cornell in 2002, two years after her older brother, Andrew J. Carlson, and two years before her younger brother, Mark A. Carlson. In 2002, Jane began her dissertation in the Department of Biological Sciences at Louisiana State University, under the supervision of Dr. Kyle E. Harms. Jane learned Spanish while doing her field work in Costa Rica and is currently learning French in Louisiana. She hopes these skills will someday be useful, namely for travel in France and Spain to study the genus *Lavandula*, which has female-biased nectar production and is pollinated by bees.