

# The relationship between nectar spur curvature in jewelweed (*Impatiens capensis*) and pollen removal by hummingbird pollinators

Steven E. Travers, Ethan J. Temeles, and Irvin Pan

**Abstract:** Floral nectar spurs are hypothesized to have had a major role in the evolution of floral diversity and plant-pollinator coadaptation. We examined variation in the degree of nectar spur curvature in two species of jewelweed (*Impatiens capensis* and *Impatiens pallida*) pollinated by different sets of pollinators. To distinguish between adaptive and nonadaptive explanations for between-species differences in curvature, we determined the relationship between spur curvature and pollen removal, which is one estimate of male reproductive success. Spur curvature exhibited considerable variation both within and among three populations, with spur angles ranging from 0° to 297°. A greenhouse experiment determined that spur curvature of *I. capensis* flowers has a broad-sense heritability of 0.636. Laboratory experiments indicated that flowers having recurved spurs deposit significantly more pollen grains on hummingbird visitors than flowers having perpendicular spurs, apparently as a result of greater contact between the androecium of curve-spurred flowers and the upper bill of hummingbirds. We also found a significant relationship between spur curvature and flower length, suggesting a developmental link between the two traits. We discuss the degree of spur curvature in bird-pollinated *I. capensis* as a function of both adaptive evolution and developmental constraint.

**Key words:** pollination, nectar spurs, hummingbirds, *Impatiens capensis*, *Impatiens pallida*, pollen export, floral morphology, floral polymorphisms.

**Résumé :** Les éperons à nectar des fleurs ont probablement eu un rôle majeur dans l'évolution de la diversité florale et la co-adaptation des pollinisateurs de plantes. Nous avons examiné la variation du degré de courbure de l'éperon floral chez deux espèces d'impatiences (*Impatiens capensis* et *Impatiens pallida*) pollinisées par différents ensembles de pollinisateurs. Pour discerner les explications adaptatives des explications non adaptatives rendant compte des différences inter-spécifiques de la courbure, nous avons déterminé la relation entre la courbure de l'éperon et une estimation du succès reproductif mâle, soit le prélèvement de pollen. La courbure de l'éperon montre une variation considérable, aussi bien entre qu'à l'intérieur de trois populations, angle des éperons allant de 0° à 297°. Une expérience en serre a permis de déterminer que la courbure de l'éperon, chez les fleurs de *I. capensis*, montre une héritabilité au sens large de 0,636. Des expériences de laboratoire indiquent que les fleurs pourvues d'éperons recourbés déposent significativement plus de grains de pollen sur les oiseaux-mouches visiteurs que les fleurs ayant des éperons perpendiculaires, apparemment suite à un contact plus étroit entre l'androcée des fleurs à éperons courbés et la partie supérieure du bec des oiseaux-mouches. Nous avons également perçu une relation significative entre la courbure de l'éperon et la longueur des fleurs, ce qui suggère un lien développemental entre ces deux caractères. Nous discutons le degré de courbure de l'éperon chez *I. capensis*, fécondé par les oiseaux, en fonction à la fois de l'évolution adaptative et de la contrainte développementale.

**Mots clés :** pollinisation, éperons à nectar, oiseaux-mouches, *Impatiens capensis*, *Impatiens pallida*, exportation de pollen, morphologie florale, polymorphismes floraux.

## Introduction

The morphological traits of flowers can have important effects on the ability of an individual plant to make genetic

contributions to future generations. In animal-pollinated plant species, the morphology of flowers can influence both the relative attractiveness of flowers to pollinators and the efficiency with which pollen is carried to and from flowers when they are visited (e.g., Schemske and Horvitz 1984; Herrera 1987; Campbell et al. 1996). More is known about what floral characters influence pollinator visitation to plants than about how morphological traits influence pollen export and deposition (Harder and Barrett 1996; Campbell et al. 1996).

One floral structure that is thought to have had a strong influence in shaping the evolution of plant and pollinator diversity is the nectar spur (Hodges and Arnold 1995). Nectar

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S.E. Travers,<sup>1</sup> E.J. Temeles, and I. Pan. Department of Biology, Amherst College, Amherst, MA 01002, U.S.A.

<sup>1</sup>Corresponding author. Present address: Department of Biology, The Pennsylvania State University, University Park, PA 16802, U.S.A. (e-mail: [set10@psu.edu](mailto:set10@psu.edu)).

spur length and colour can determine the type and quantity of pollinators attracted to the plant (Grant 1952; Miller 1981; Hodges and Arnold 1994). Adaptation of bee pollinators to plants and their nectar spurs is suggested by the correlation between spur length and tongue lengths of visiting bees (Grant and Temeles 1992; Inouye 1980). Plant fitness within a population has even been correlated with spur length (Nilsson 1988). We report here on a study designed to examine the relationship between nectar spur morphology of a hummingbird-pollinated plant and pollination by hummingbirds. In particular, we were interested in determining the extent to which nectar spur curvature in *Impatiens capensis* represents an adaptation to hummingbird pollination.

Spur length and curvature vary enormously in the *Impatiens* genus worldwide (Grey-Wilson 1980). In two North American species, *Impatiens pallida* and *I. capensis*, nectar spur characteristics apparently are associated with pollinator differences. Flowers of *I. pallida* have much smaller nectar spurs (approximately 5 mm in length; Schemske 1978) than *I. capensis*, with less volume for nectar, and are pollinated almost exclusively by bees (Wood 1975; Randall 1988; Wilson 1995). In contrast, flowers of *I. capensis* have longer nectar spurs (up to 12 mm in length; Lavery and Plowright 1985) with more volume for nectar (as much as 5–6  $\mu$ L standing crop; Heinrich 1976; Lavery and Plowright 1985) and are pollinated by both ruby-throated hummingbirds (*Archilochus colubris*) and bees (Heinrich 1976; Bertin 1982; Lavery and Plowright 1985). In African species of *Impatiens*, bird-pollinated species tend to have very long, recurved spurs, whereas bee-pollinated species tend to have shorter spurs oriented vertically relative to the flower tube (Grey-Wilson 1980). The orientation of nectar spurs also differs between the two North American *Impatiens* species. Nectar spurs of *I. capensis* tend to curve forward towards the opening of the flower. In contrast, the spurs of *I. pallida* are generally perpendicular to the long axis of the flower.

In considering the evolution of nectar spur orientation in *Impatiens*, we have developed adaptive and nonadaptive hypotheses. First, increased spur curvature in bird-pollinated *I. capensis* may be the result of a positive relationship between spur curvature and reproductive success when birds are the pollinators. For example, individual plants may export or receive more pollen from bird pollinators when nectar spurs are curved than when they are oriented straight back. Alternatively, there may be no fitness effects of spur curvature. The differences in spur orientation between the species could be due to developmental constraints, whereby the longer flowers or longer spurs of *I. capensis* may automatically result in more curved spurs because of a limited number of developmental patterns for nectar spurs.

We examined patterns in spur curvature in bird- and bee-pollinated *Impatiens* species and conducted manipulative experiments on *I. capensis* to estimate the influence of spur curvature on pollen removal. Specifically, we addressed the following questions: (i) How variable are curvatures of nectar spurs of *I. capensis* in field populations? (ii) How does variation in nectar spur curvature in hummingbird pollinated *I. capensis* compare with spur curvature in bee-pollinated *I. pallida*? (iii) What is the broad-sense

heritability of nectar spur curvature (i.e., could this trait theoretically respond to selection)? (iv) Is there a relationship between nectar spur curvature and pollen deposition on bird visitors? (v) Is spur curvature an allometric function of flower size or spur size?

## Materials and methods

### Spur curvature censuses

We censused new flowers on *I. capensis* individuals growing in three natural populations to describe the variation in spur curvature infield conditions. The three populations (Campus, Bay Road, 116) were in Amherst, Franklin County, Massachusetts, and were approximately 0.2–6.4 km apart. Each population contained between 200 and 1000 individual plants. Based on personal observations, we estimated that most pollination of *I. capensis* at the Bay Road population was due to hummingbirds, while pollination at the 116 and Campus populations was due to bumblebees.

Between July 3 and September 1, 1998, the populations were censused at weekly intervals. Floral life of *I. capensis* is less than 4 days (Bell et al. 1984), so by spacing our censuses a week apart we avoided repeat censuses of the same flowers. At each of these censuses we recorded the spur angle of all recently opened flowers in the population. Spur curvature was measured as the angle between an imaginary line emerging from the back end of the flower parallel to the midpoint of the flower throat and the main axis of the nectar spur (Fig. 1). Thus, a flower with a spur that orients straight back from the flower has a curvature of zero, while a spur that curves straight forward underneath the flower has a curvature of 180°.

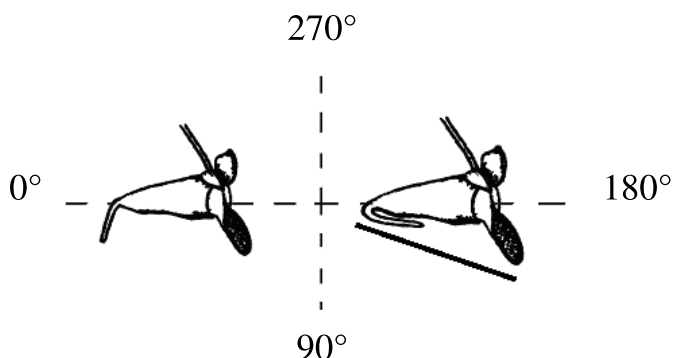
### Herbarium survey of two *Impatiens* species

We used herbarium specimens of two *Impatiens* species to document the variation in floral characteristics of the two species. All dried plant specimens examined are housed in the herbarium at the University of Massachusetts, Amherst, Mass. We measured flower characteristics on 106 separate specimens of *I. capensis* and 56 specimens of *I. pallida*, for a total of 162 specimens. These specimens were collected over a 183-year period from 1817 to 1999. The *I. capensis* samples were collected in 18 American states and five Canadian provinces, whereas those of *I. pallida* came from 15 states and one province. On each specimen we measured a single randomly chosen flower for the following variables: flower length, nectar spur length, and spur curvature. Flower length is equal to the distance between the base of the nectar spur and the distal most point of the lower flower lip (Fig. 1).

### Heritability estimates

To examine whether there was a genetic basis to spur curvature, we conducted repeatability analyses using the census data, and a separate heritability study using greenhouse plants. Repeatability is a measure used in quantitative genetics studies to assess the proportion of variance in a trait that occurs among, rather than within, individuals. Repeatability,  $r$ , is given by the formula  $r = s_A^2 / (s^2 + s_A^2)$ , where  $s_A^2$  is the among-groups variance component and  $s^2$  is the within-groups variance component.

**Fig. 1.** Measurement of floral spur angle as the deviation from a line proceeding posteriorly through the middle of the flower throat. The spur angles depicted above are 85° (perpendicular) and 175° (recurved) for the left and right flowers, respectively. The solid bar indicates the distance measured for flower length.



Repeatability analyses are a first step in determining whether a quantitative trait has a heritable basis, and provide an upper boundary on the heritability of a trait (values of heritability range from 0–1). Calculations of repeatabilities followed procedures outlined in Lessells and Boag (1987), and 95% confidence intervals for repeatability estimates followed procedures in Becker (1984).

We estimated heritability of spur curvature in *I. capensis* through the use of parent–offspring analysis on greenhouse plants. We established the parental generation in the greenhouse by transplanting young seedlings from a single population (Bay Road) into plastic pots prior to flowering. The seedlings were planted in standard greenhouse potting soil in the pots and transferred to the greenhouse, where they were watered daily and fertilized once a week. We collected a total of 35 seedlings for the parental generation.

Because *I. capensis* also produces cleistogamous flowers, it was possible to collect seed for the offspring generation from fruits produced by the cleistogamous flowers within a plant. All of the offspring grown for the following generation were thus produced by selfing. Seeds collected from the parental plants were then used to grow multiple offspring per parent for measures of spur curvature and the estimation of heritability. We followed the seed germination and seedling growth protocol used by Donohue and Schmitt (1999).

We measured spur curvature in both the parental and offspring generations using the method described above. In both the parental and offspring generation, between 1 and 5 flowers were measured per plant. The mean measurement of these flowers per parental plant was used in the parent–offspring regression analysis. The curvature values for the offspring generation were based on the mean of spur curvature estimates among the offspring of a particular parental plant ( $n = 2\text{--}5$  individuals/family).

To estimate the broad-sense heritability of spur curvature we regressed offspring estimates of spur curvature on the curvature estimates of their parent plants (Falconer 1989). Because the offspring were full sibs produced by selfing, we calculated heritability of spur curvature as the slope of the regression divided by  $2r_{xy}$ , where  $r_{xy}$  is a measure of the relatedness of parents and offspring (Smith and Kinman 1965).

### Pollen removal experiment

In an earlier study (Hurlbert et al. 1996), we found that nectar spurs had a major role in the pollination mechanism of *I. capensis* flowers. Accordingly we conducted a laboratory experiment to examine whether spur curvature influenced the amount of pollen removed by hummingbirds from male-phase *I. capensis* flowers. Captive hummingbirds (*A. colubris*) were allowed to visit *I. capensis* flowers assigned to one of two spur curvature categories with different degrees of spur curvature, perpendicular and recurved (Fig. 1). Perpendicular spurs were bent at an approximately 90° angle to the axis of the floral tube (range 45°–110°). Recurved spurs were bent completely back towards the flower opening, parallel to the axis of the floral tube (range 152°–210°). We then measured the amount of pollen deposited onto the hummingbird pollinators and compared the number of pollen grains deposited from recurved versus perpendicular flowers.

Six male hummingbirds were used in the laboratory experiment. All six birds were captured in Amherst, Franklin County, Mass., at least 5 days before experimental trials. The birds were housed in 2 m × 2 m × 1.5 m mesh tents and fed an ad libitum diet of Nektar Plus (Nekton, U.S.A.) from plastic feeders for 3 to 5 days to acclimate them to the laboratory setting. The birds were then trained to feed from jewelweed flowers by offering each bird a jewelweed flower filled with 40% sucrose solution (w/w). Once they had fed 10 times from jewelweed flowers, the birds were subjected to experimental trials.

An experimental trial began by placing a bird in a 2.5 m × 2.25 m × 1.5 m observation room in isolation with a single stem and flower of jewelweed. The plant cuttings were collected the morning of experimental trials between 05:30 and 07:00. A cutting consisted of a single male-phase flower, newly or partially opened, and an approximately 10-cm section of main stem to which the flower was connected. The cut stems were placed in individual polyethylene centrifuge tubes (2 mL) filled with water. Just prior to an experimental trial, all of the leaves on the cutting were removed and the cutting in its tube was attached to a wooden stand in the observation room. To control for variation in nectar volume among flowers, which could influence handling time and pollen removal (Temeles and Rankin 2000), we removed all of the nectar from the spur of the flower using a micropipetter fitted with a gel-loading pipette tip. We then replaced the nectar with 2 µL of 40% sucrose solution (2 µL of 40% sucrose solution represents the average standing crop and concentration of nectar found in flowers within the Amherst area; Pan 1999). We used a video camera (Panasonic 5100HS), focused on the flower, to measure the duration of a hummingbird visit to the flower. During the trial, we could observe the bird both through a one-way mirror and on the video monitor connected to the camera. After a bird visited the flower once, the trial was concluded. The bird was then captured and the pollen was washed from its head and bill, as described in Temeles and Pan (2002), to determine the amount of “passive pollen placement” (Inouye et al. 1994). The total number of pollen grains on each bird was estimated using the subsampling technique described in Hurlbert et al. (1996).

Each bird was exposed to 10 pairs of curved and perpen-

dicular flowers. The order of presentation of curved versus perpendicular flowers within a given replicate was alternated such that one bird first visited perpendicular and then curved flowers, while another bird first visited recurved and then perpendicular flowers. By alternating the order of presentation we controlled for any effects of presentation order on bird behaviour.

We analyzed each hummingbird visit to a recurved or perpendicular flower separately using Peak Motus Software (Peak Performance Technologies, Inc.) developed for frame-by-frame analysis of videotape. With this software we measured handling time (defined as the elapsed time from the moment the bird's bill entered the flower to the moment the bill left the flower) accurately to one hundredth of a second. By dividing the amount of pollen deposited on the bird during a visit by handling time, we obtained the efficiency of pollination (number of grains deposited on the bird/second of visiting time) for each visit. Because we had previously observed flowers oscillating horizontally when birds visited them (Hurlbert et al. 1996), we also measured the number of flower oscillations per visit. Each oscillation corresponds to an extension of the bird's tongue into the flower's nectar spur and displaces the flower by one flower length (Temeles and Ewald 1999). We used paired *t*-tests to compare handling times, pollen loads, pollination efficiencies, and oscillation number between recurved and perpendicular flowers. For each variable, we used the mean of the 10 visits to each flower type for each of the six birds in the experiment. Significance levels for specific comparisons were adjusted for an overall error rate of 0.05 using Bonferroni sequential tests (Rice 1989).

## Results

### Variation in spur curvature within and between populations

Flowers of *I. capensis* in field populations are extremely variable in the curvature of nectar spurs. The results of surveys of spur curvature summarized in Table 1 indicate that spur curvature varied over the widest range (from 0° to 297°) in population 116. The majority of flowers had spur curvatures measuring between 80° and 190° in all three populations (Figs. 2A, 2B, and 2C), whereas the pooled mean of all the populations was 153.3°. Two of the populations (116 and Bay Road) had distinct bimodal distributions with peaks at approximately 90° and 180° (Figs. 2B and 2C).

### Heritability of spur curvature

Repeatability estimates for the three populations of *I. capensis* in this study ranged from 0.398 to 0.461 (repeatability estimates (in italics) and 95% confidence intervals, population 116: 0.389 < 0.450 < 0.520; Campus: 0.298 < 0.398 < 0.500; Bay Road: 0.361 < 0.461 < 0.570; analyses performed on the population data in Fig. 2). None of the 95% confidence intervals for the repeatability estimates overlapped zero, suggesting the potential for a heritable genetic component for spur curvature.

The regression of offspring spur curvature on parental plant spur curvature indicated that the slope of the relationship was significantly different from zero ( $r^2 = 0.691$ ,  $n = 7$ ,  $b = 1.271$ ,  $SE = 0.38$ ,  $F = 11.17$ ,  $p < 0.05$ ). Based on these

**Table 1.** Descriptive statistics for spur curvature in three field populations of *Impatiens capensis* (see Fig. 2).

| Population | <i>n</i> | Curvature (degrees) |      |             |
|------------|----------|---------------------|------|-------------|
|            |          | Min.                | Max. | Mean ± SE   |
| Campus     | 616      | 2                   | 270  | 162.4 ± 1.2 |
| Bay Road   | 508      | 10                  | 215  | 154.4 ± 1.4 |
| 116        | 1559     | 0                   | 297  | 149.5 ± 1.0 |
| Pooled     | 2683     | 0                   | 297  | 153.3 ± 0.7 |

**Note:** Differences in mean spur curvature between populations are significant for all comparisons ( $p = 0.05$  overall, one-way ANOVA with Tukey multiple comparisons).

results, the estimate of heritability of spur curvature in the greenhouse population studied here is  $b/(2r_{XY}) = 0.636$ . The range of spur curvatures within an individual plant was, on average, 55° ( $SE = 4.3$ ,  $n = 67$ ).

### Herbarium survey of allometric relationships

Regression analyses indicated that there was a significant positive relationship between flower size and spur curvature for both *I. capensis* ( $df = 104$ ,  $F = 6.54$ ,  $p = 0.012$ ,  $R = 0.245$ ) and *I. pallida* ( $df = 72$ ,  $F = 3.99$ ,  $p = 0.049$ ,  $R = 0.230$ ; Fig. 3). Moreover, the slopes of the best-fit lines for the data from each species were nearly identical (*I. capensis*:  $y = 2.77x + 122.0$ ; *I. pallida*:  $y = 2.84x + 86.3$ ). However, the y-intercept for the regression of spur curvature on flower length for *I. pallida* was lower than the intercept for *I. capensis*. The mean spur curvature for *I. capensis* ( $167.2^\circ \pm 2.8^\circ$ , mean ± SE) was significantly different from the mean spur curvature of *I. pallida* ( $129.1^\circ \pm 3.3^\circ$ ) according to the results of a student's *t*-test ( $df = 177$ ,  $t = 79.28$ ,  $p < 0.0001$ ). Spur length was, on average, greater for *I. capensis* than for *I. pallida*. There was no relationship between spur curvature and spur length in either species (*I. capensis*:  $df = 104$ ,  $F = 0.300$ ,  $p > 0.50$ ; *I. pallida*:  $df = 72$ ,  $F = 0.932$ ,  $p > 0.30$ ).

### Effect of spur curvature on pollen removal by hummingbirds

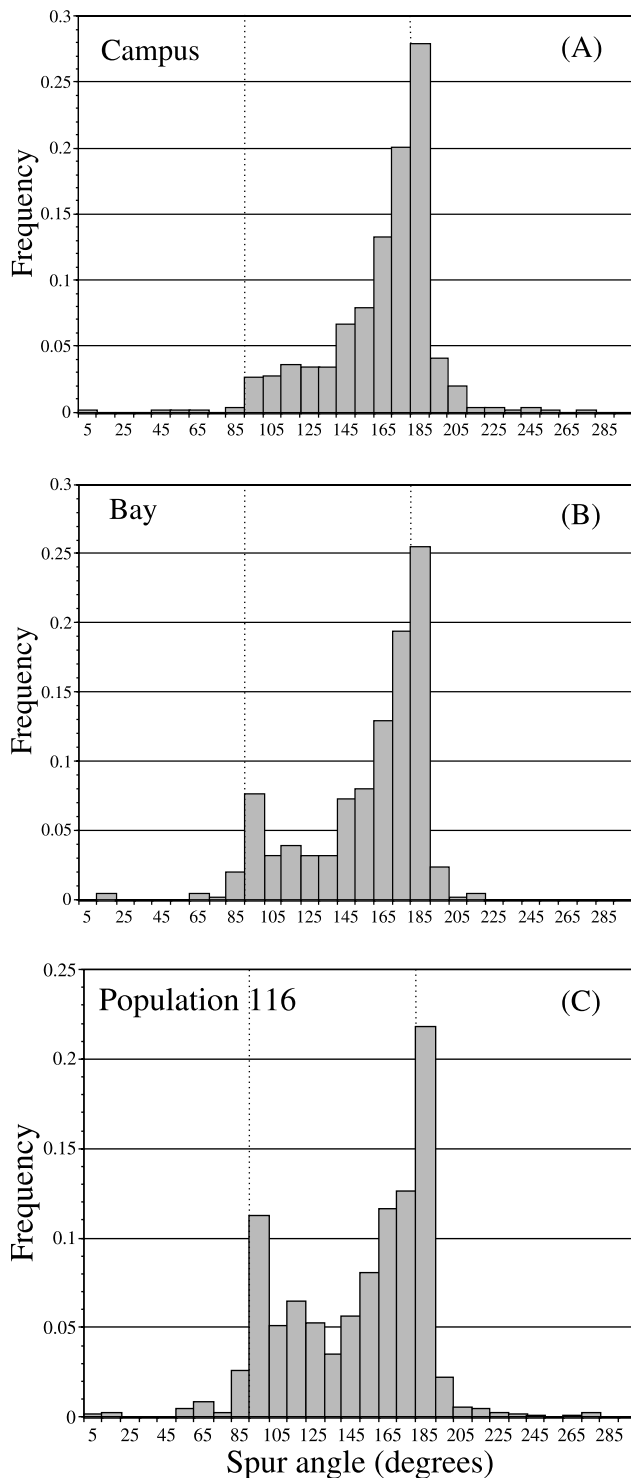
Hummingbirds removed 1.75 times more pollen per visit from flowers with recurved nectar spurs than from flowers with perpendicular nectar spurs (Table 2). Handling times of birds were 1.13 times longer at curve-spurred flowers than at perpendicular-spurred flowers, but this difference was not statistically significant. As a result of the difference in pollen removal between recurved and perpendicular flowers and the lack of difference between the flower types in hummingbirds' handling times, pollen removal efficiency was significantly higher (by a factor of 1.44) at curve-spurred flowers than at perpendicular-spurred flowers (Table 2). The number of oscillations in the horizontal plane was significantly higher for curve-spurred flowers than for perpendicular-spurred flowers (Table 2).

## Discussion

### Between-species differences

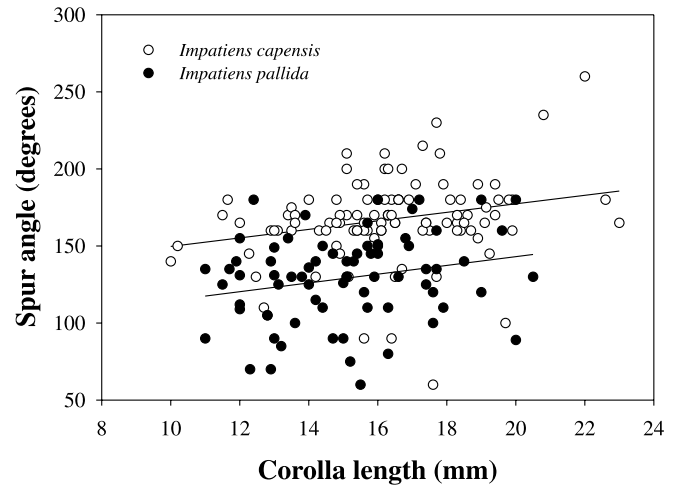
We found variation in the degree of curvature of *I. capensis* nectar spurs in both field populations and herbarium specimens. Spurs ranged in their orientation from nearly straight back to curling over 180° and pointing upward

**Fig. 2.** Frequency distributions of floral spur angles for three populations of *Impatiens capensis*. The number of spurs measured in each patch were as follows: (A) Campus,  $n = 616$ ; (B) Bay Road,  $n = 546$ ; (C) 116,  $n = 1559$ . Values on the  $x$ -axis represent bin midpoints. Dotted lines indicate  $90^\circ$  and  $180^\circ$ .



(field: mean =  $153.3^\circ$ ; herbarium: mean =  $167.2^\circ$ ). In contrast, bee-pollinated *I. pallida* had smaller spurs that were generally perpendicular to the main axis of the flower (mean =  $129.1^\circ$ ).

**Fig. 3.** Spur angle as a function of flower length for *Impatiens capensis* and *Impatiens pallida*. Best-fit lines were determined by least-squares regression analysis.



In considering the evolution of spur orientation in *Impatiens*, we have examined both adaptive and nonadaptive explanations. Our results support the conclusion that spur curvature reflects both a response to selection and the influence of nonadaptive developmental processes. We found a positive relationship between flower length (but not spur length) and spur curvature in both species. However, this link between traits is unlikely to be the sole explanation for increased spur curvature in *I. capensis*, which does have longer flowers than *I. pallida* on average (Schemske 1978). Despite nearly identical slopes of the best-fit lines for the relationships between curvature and length for the two species, the y-intercepts of those lines were different. Nectar spurs were more curved on *I. capensis* than on *I. pallida* for flowers of the same length. Differences in the y-intercepts of the best-fit lines could be explained by a selective advantage of spur curvature in the bird-pollinated species. Nectar spur morphology had a significant impact on the amount of pollen being removed by a plant per hummingbird visit. All else being equal, an *I. capensis* plant with a recurved spur is expected to remove more pollen through visiting hummingbirds than a plant with a perpendicular spur (Table 2). We observed that flowers with recurved nectar spurs appeared to have greater contact between the androecium containing the pollen and the hummingbird visitor during the course of nectar extraction relative to perpendicular spurred flowers. Flowers with recurved spurs oscillated in the horizontal plane much more noticeably with the tongue movements of the bird relative to perpendicular spurred flowers (Table 2), perhaps as a result of increased friction of the recurved spur on the retracting tongue inside the recurved spur.

Intraspecific variations in floral morphology have previously been shown to influence the efficiency of pollination by birds. Campbell et al. (1991, 1996) found that *Ipomopsis* floral morphology influenced pollen removal, not through increased pollinator attraction and increases in visits, but by increasing the efficiency of pollen transfer to the pollinator and other flowers. Broader flowers allowed birds to probe for nectar more deeply and thus increase their contact with anthers. Similarly, recurved nectar spurs in jewelweed ap-

**Table 2.** Effect of spur curvature on pollen export, handling time, pollination efficiency, and horizontal flower oscillations during hummingbird visits.

| Spur curvature        | <i>n</i> | Pollen grains exported<br>(mean $\pm$ SE) | Handling time,<br>s/visit (mean $\pm$ SE) | Pollination<br>efficiency, grains/s<br>(mean $\pm$ SE) | Horizontal oscillations,<br>no./visit (mean $\pm$ SE) |
|-----------------------|----------|---|---|--|---|
| Recurved              | 6        | 5209 $\pm$ 1065                           | 1.60 $\pm$ 0.20                           | 3397 $\pm$ 640   | 4.62 $\pm$ 0.90                                       |
| Perpendicular         | 6        | 2969 $\pm$ 694                            | 1.41 $\pm$ 0.17                           | 2348 $\pm$ 468   | 2.73 $\pm$ 0.85                                       |
| Paired <i>t</i> -test |          |   |   |  |   |
| <i>t</i>              |          | 3.36                                      | 1.81                                      | 3.55   | 6.16  |
| <i>p</i>              |          | 0.02*                                     | 0.129                                     | 0.016*   | 0.002*  |

\**p* < 0.05, sequential Bonferroni tests.

pear to increase the contact between androecium surfaces and a bird's bill by influencing the mechanical interaction between a flower and a feeding bird. We know from our breeding experiment that there is significant broad-sense heritability for spur curvature and little evidence that environmental conditions play a large role in determining the expression of this trait. Thus, depending on the relationships between seed paternity and pollen dispersal, and between pollen dispersal and pollen deposition on bird bills, selection through male reproductive success may favor recurved nectar spurs in jewelweed populations visited by hummingbirds.

Our study only measured pollen deposition on bird bills and did not directly measure the male component of fitness or the number of seeds sired by pollen from recurved and perpendicular flowers. Evidence from other flowering plant species, however, suggests that pollen removal is a good predictor of paternity (Hainsworth et al. 1985; Campbell 1991; Galen 1992; Campbell et al. 1996). We also examined pollen removal exclusively and did not examine pollen deposition onto stigmas. It is possible that the increased contact between anthers and birds associated with spur curvature is mirrored by increased contact with stigmas, because the mature stigma of a jewelweed flower is located near the androecium. Future considerations of spur curvature would benefit from measuring its impact on female reproductive success and pollen limitation.

### Variation within species

If spur curvature is directly related to plant reproductive success and fitness in bird-pollinated *Impatiens* species, then selection should reduce variation around the population mean for spur curvature. Indeed, strong selection for recurved spurs could lead to canalization of the curvature phenotype. However, our field and herbarium surveys indicated that there is a great deal of variation in the degree of spur curvature in *I. capensis*.

To understand the role of selection and constraint in determining the range of spur curvatures in *I. capensis*, it is useful to consider the development of the spur. In the genus *Impatiens*, the spur develops as a basal appendix of the median calyx segment with a forward curvature. The presence of this developmental pattern indicates that initial curving of the spur is annulled in those species that ultimately have perpendicular nectar spurs such as *I. pallida*. Thus, perpendicular spurs are not the result of developmental constraint, but rather may indicate some selective advantage for perpendicular orientation. The shared characteristic for both *I. pallida*

and *I. capensis*, which both have some perpendicular-spurred morphs, is that they are pollinated either partially or completely by insects (Rust 1977a, 1977b, 1979). There is some evidence that insect pollination results in improved reproductive success for perpendicular- versus recurved-spur flowers. In an earlier study on *I. capensis*, Pan (1999) found that nectar robbery by bees rose with increasing spur curvature, perhaps because bees could more effectively land on spurs that curved more than 100° and made an effective landing pad. If nectar robbery decreases bird visitation, pollen deposition, and pollen removal, then selection would favor spurs that are perpendicular to the main axis of the flower. In another study (Temeles and Pan 2002), pollen removal from *I. capensis* flowers was lower for robbed versus unrobbed flowers.

Given that insect and bird pollination can favor different spur morphology, the extraordinary range of spur curvatures in *I. capensis* could be the result of this species having both types of pollinators servicing its flowers. Thus, active selection may maintain both curved- and perpendicular-spurred individuals in *I. capensis* populations through variation in reproductive success. Indeed, our surveys indicated that distributions of spur curvature from natural populations were bimodal, with peaks at 90° and 180°. Alternatively, *I. capensis* and *I. pallida* may share a perpendicular-spurred ancestor that was insect-pollinated, making perpendicular spurs a symplesiomorphy in *I. pallida* and *I. capensis*. In this scenario, even weak selection for spur curvature in *I. capensis* could result in a wide range of spur curvatures for *I. capensis* but not *I. pallida*.

Another explanation for such a wide range of spur curvature in *I. capensis* is that curvature may vary within populations of *I. capensis* because of environmental effects. Despite the significant broad-sense heritability of spur curvature, the trait may still have a maternal environmental source of variation (Falconer 1989). We know that larger flowers produce spurs that are more curved, which suggests that any environmental effect on flower size (e.g., soil nutrition, which influences plant size) could indirectly influence spur curvature. Environmental effects on spur curvature could weaken the response to selection due to bird pollination. Ultimately, the degree of spur curvature in bird-pollinated species with nectar spurs may depend on the mechanical interaction between the morphology of the flowers and the pollinator and on environmental and developmental sources of variation in the phenotypic characteristics of the nectar spur.

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