Effect of the lower lip of *Monarda didyma* on pollen removal by hummingbirds

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**Abstract:** We examined the effect of the lower lip of *Monarda didyma* L. on pollen removal by ruby-throated hummingbirds (*Archilochus colubris* L.) through comparisons of hummingbird visits to natural, lipped flowers versus those that had their lower lip experimentally removed. Birds missed the openings of lipless flowers more often than lipped flowers and thus spent more time attempting to insert their bills into them. Once their bills entered the floral tube, handling times of hummingbirds were significantly longer at lipped than at lipless flowers. Contact durations with anthers and amounts of pollen removed by hummingbirds were significantly greater at lipped than at lipless flowers. Video-tapes indicated that lips enhance pollen removal by slowing a hummingbird’s bill insertion into the floral tube, which increased the amount of time that a bird was positioned under a flower’s anthers. Unlike the conventional view of hummingbird--flower evolution, which argues for the loss or reduction of lips, our results suggest that lower lips may be maintained in some hummingbird flower species, because they are preadaptations that act as nectar guides for hummingbirds and positioners for optimum pollen removal, in addition to possibly serving as landing platforms or attractants for secondary pollination by bees.

**Key words:** *Monarda didyma*, pollination, flower morphology, hummingbirds.

**Résumé :** Les auteurs ont examiné les effets de la lèvre inférieure du *Monarda didyma* L. sur la libération du pollen par l’oiseau-mouché à gorge rubis (*Archilochus colubris* L.) en comparant les visites d’oiseaux-mouches sur des fleurs naturelles, ayant leurs lèvres intactes, avec les visites sur des fleurs dont la lèvre a été enlevée expérimentalement. Les oiseaux manquèrent les ouvertures des fleurs sans lèvre plus souvent que celles qui ont leurs lèvres, et ainsi passent plus de temps à essayer d’introduire leur bec dans celles-ci. Une fois que leur bec est introduit dans le tube floral, le butinage par les oiseaux-mouches sont significativement plus longs sur les fleurs ayant leurs lèvres, que celles qui ne l’ont plus. Les durées de contact avec les anthères et les quantités de pollen libérées par les oiseaux-mouches sont significativement plus importantes chez les fleurs ayant leur lèvre intacte que sur celles qui ne l’ont plus. Les bandes vidéo indiquent que les lèvres favorisent la libération du pollen en ralentissant l’insertion du bec des oiseaux-mouches dans le tube floral, ce qui augmente la durée pendant laquelle l’oiseau est placé sous une anthère de la fleur. Contrairement aux vues conventionnelles suggérant que l’évolution oiseau-mouché – fleur encourage une réduction de la lèvre, ces nouveaux résultats suggèrent que la lèvre inférieure peut être maintenue chez certaines espèces de fleurs associées aux oiseaux-mouches, parce qu’elles sont des préadaptations qui agissent comme guides à nectar pour ces oiseaux et favorisent leur positionnement de manière à optimiser la libération du pollen, en plus de servir possiblement de plate-forme d’atterrissage pour des pollinisateurs secondaires tels que les abeilles, ou en les attractant.

**Mots clés :** *Monarda didyma*, pollinisation, morphologie florale, oiseaux-mouches.

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**Introduction**

A major organizing principle of pollination biology is the concept of pollination syndromes (e.g., Faegri and van der Pijl 1979; Proctor et al. 1996). Pollination syndromes are suites of floral traits proposed to reflect adaptation to pollination by animal groups such as birds, bats, bees, butterflies, and moths (Faegri and van der Pijl 1979).

One of the best examples of a pollination syndrome is the association between birds and bird-pollinated flowers, referred to as the “ornithophilous syndrome” (Faegri and van der Pijl 1979). Many pollination biologists, including Darwin, have noted that flowers pollinated by birds tend to be red in colour, produce copious, odorless nectar, and have reduced petals and long floral tubes that match the bills of their bird pollinators (Darwin 1876; Grant and Grant 1968; Faegri and van der Pijl 1979; Proctor et al. 1996). The idea that bird-pollinated flowers are long red tubes that correspond to the size and shape of birds’ bills has been presented as a major aspect of bird–flower coevolution in textbooks and reviews for decades (Faegri and van der Pijl 1979; Johnsgard 1983; Gould and Keeton 1996; Proctor et al. 1996; Raven and Johnson 1999).

Recent studies reveal that flower pollination by birds may involve more than a simple match between bill and flower dimensions. Tongue pollination, for example, was assumed to be restricted to insects until Pauw (1998) demonstrated...
that some flowers pollinated by sunbirds deposit pollen onto the birds’ tongues. Similarly, the flexible pedicels of some bird-pollinated flowers were viewed as adaptations to prevent the ovaries of flowers from being pierced by birds’ probing bills (Grant and Grant 1968), but experiments indicate that the flexible pedicels of some hummingbird-pollinated flowers cause the flowers to move during hummingbird visits and that these movements enhance polination (Hurlbert et al. 1996). These studies suggest that our knowledge of the features comprising a given pollination syndrome may be incomplete and that the roles of some floral traits in the pollination process (e.g., flexible pedicels) may have been incompletely defined.

The purpose of this paper is to report on findings concerning the role of flower lips in hummingbird pollination. A long-standing view in the evolution of bird-pollinated flowers is that lips and petals around the flower opening are either lost or reduced, because birds are too large to alight on these structures and (or) because loss of such structures promotes specialization on birds by discouraging insect visitors (Grant and Grant 1968; Faegri and van der Pijl 1979). Many ornithophilous flowers retain lips and petals, however, and these structures have been viewed as either transitional states in the evolution from bee- to bird-pollinated flowers or as landing platforms that facilitate insect-pollination of flowers that are pollinated by both birds and insects (Grant and Grant 1968).

In a previous study, Temeles (1996) determined that ruby-throated hummingbirds (Archilochus colubris L.) occasionally missed the openings of narrow flowers with their bills and tongues; they made “insertion” errors. These insertion errors increased a bird’s feeding time at a flower, because the bird had to reposition itself following an error to extract its bill into the flower to correctly feed. Based on this finding, Smith et al. (1996) hypothesized that lips and petals may be retained on some flowers pollinated by hummingbirds because they are preadaptations that reduce the frequency of insertion errors. Subsequent experiments on Monarda didyma L., and Monarda fistulosa L., hummingbird-pollinated flowers lacking visible or ultraviolet nectar guides (Whitten 1981; Smith et al. 1996), and on artificial flowers varying in size and morphology, supported this hypothesis by demonstrating that the lower lip of flowers can function as a guide to reduce the frequency of insertion errors (Smith et al. 1996).

During their experiments, Smith et al. (1996) discovered that lips significantly increased a hummingbird’s feeding time once its bill had entered the flower (= handling time). For M. didyma, the increase in handling time at lipped flowers was considerable: hummingbirds’ handling times averaged 20% longer at flowers whose lips had been experimentally removed than at normal flowers (Smith et al. 1996). Because handling time is significantly related to the duration of another contact (Hurlbert et al. 1996), lipped flowers may benefit from longer handling times relative to lipless flowers; this assumes that pollen removal is related to another contact duration.

In the present paper, we examine the effect of the lower lip of M. didyma flowers on pollen removal by ruby-throated hummingbirds. We consider three hypotheses for how lower lips could enhance pollen removal. First, Smith et al. (1996) suggested that the lower lip may misdirect the bird from the center of the flower opening, thereby causing the bird to be misaligned with the floral tube. Because of this misalignment, a bird may spend more time adjusting its position once its bill enters the floral tube, which could increase another contact and pollen removal, assuming that these variables are related to handling time (see Hurlbert et al. 1996; Smith et al. 1996). Alternatively, the lower lip may act as an obstacle that causes a hummingbird to either slow its bill insertion into the floral tube, or alternatively to hold its body farther back from lipped flowers than lipless flowers during the feeding visit. In either case, the lower lip would cause a hummingbird’s bill tip to be farther from the nectar source, resulting in longer tongue extension, handling times, and possibly longer another contact and greater pollen removal (Hainsworth and Wolf 1976; Montgomerie 1984; Temeles and Roberts 1993). Following our analysis of the effect of flower lips on pollen removal by hummingbirds, we discuss the role of lips and petals in hummingbird–flower relationships.

Materials and methods

The birds

The experiments were conducted on 10 adult male ruby-throated hummingbirds (A. colubris) that had been captured in Shutesbury, Mass., U.S.A., from June through August 1995. Following capture, individual birds were housed in 0.92 × 0.92 × 0.75 m (height × width × depth) cages in a light-controlled room (14 h light:10 h dark cycle). Birds were fed an ad libitum diet of Nectar Plus commercial hummingbird food dissolved in distilled water (1:7 by mass).

Previous studies indicate that captive hummingbirds may increase in weight because of ad libitum feeding and lack of exercise (Smith et al. 1996). As a consequence, birds may be less motivated to perform in experiments, because they are satiated and (or) have large fat reserves. To control for this effect, birds were provided with a solution of food diluted to 25% of its normal concentration for 4 h on the evening prior to the experiment and for 1 h prior to the experiment.

The flowers

Monarda didyma is a perennial herb that ranges from New England west to Michigan, north to Quebec, and south to Georgia (Hylander 1954). It blooms from June through August (Peterson and McKenzie 1968). Flowers occur in whorls around a central stalk, and each flower has a flexible pedicel and is bilaterally symmetrical with an upper lip and a recurved lower lip. A stigma and two anthers are located underneath the upper lip and are moderately exerted. Both hummingbirds and bees (see below) have been recorded as pollinators of M. didyma, although its red colouration and long, narrow corolla suggest that it is adapted primarily for hummingbird pollination (Grant and Grant 1968; Whitten 1981; Johnsgard 1983). Whitten (1981) noted that ruby-throated hummingbirds accounted for 87.5–100% of pollinator visits to M. didyma at three sites in North Carolina and Tennessee, U.S.A., with bees (Bombus and Dialictus spp.) accounting for the remainder. Flowers of M. didyma lack colour nectar guides in both the visual and ultraviolet spectra (Whitten 1981; Smith et al. 1996). Monarda didyma is dichogamous and protandrous. Flowers are in male phase for 1 or 2 days and then advance to female phase for 1 or 2 days (Whitten 1981; personal observations).
The experiment

Birds were tested individually in a 2.11 × 1.36 × 1.48 m walk-in cage. They were given 1 day to adjust to the experimental cage before trials began. During this period, birds were trained to visit *M. didyma* flowers by placing a flower into or adjacent to the bird’s feeder. If after 2 h the bird had not fed from the flower, it was caught and hand fed from the flower until it recognized it as a food source.

Our experiment was designed to examine whether lower lips enhance pollen removal and, thus, was performed on newly dehisced, male-phase flowers. Ten plants grown from native stock by WE- DU Nurseries of Marion, N.C., U.S.A., were obtained for the experiment. Half of the flowers in this experiment were intact, lipless flowers, and half were manipulated, lipless flowers. We created lipless flowers by cutting off the lower lip of male-phase flowers from each of the 10 plants in a straight line at its junction with the opening of the corolla. To control for potential differences between individual plants that could influence pollen removal by birds, each bird was tested on one lipmed and one lipless flower from the same plant, for all 10 plants in the experiment.

Experiments were conducted from approximately 09:00 to 14:00, and each bird was tested on five lipmed–lipless flower combinations per day. Flowers were presented to birds in succession, and the order of presentation of lipmed or lipless donor flowers was staggered within and among birds to control for any effect that presentation order may have had on the birds’ behaviour. For example, if the first bird tested was given a lipmed flower and then a lipless flower, then the next bird was given flower types in reverse order (lipless flower, then lipmed flower). Each of 10 birds was videotaped at two flowers from 10 plants for a total of 200 feeding visits (100 lipmed flowers, 100 lipless flowers).

To ensure that the bird visited only the experimental flower, “hoods” from black construction paper were constructed that prevented access to all but one flower on the whorl. When necessary, flowers adjacent to the experimental flower were removed. The hood was mounted over the flowers by placing it on a stick, which was then anchored in the soil of the plant’s pot.

Because feeding times of hummingbirds vary with nectar volumes (e.g., Montgomerie 1984), the original nectar was removed from a flower prior to presenting it to a bird using small strips of filter paper measuring approximately 2 × 50 mm (Kearns and Inouye 1993). The flower was then filled with 8 μL of 20% sucrose solution (mass:mass). Following these preparations, the plant was introduced to the bird in the experimental room. The bird was allowed to make a single visit to the experimental flower, and the visit was recorded on videotape using a tripod-mounted video camera positioned approximately 60 cm from the flower. Flowers were filmed in profile, which permitted an unobstructed view of bill entry and exit as well as anther contact in both the horizontal and vertical plane.

Immediately following the visit to the experimental flower, pollen was collected from the bird’s head. The lights in the room were dimmed, the bird was captured by hand, and its bill was placed into a 1.5-mL microcentrifuge tube. The pollen was then rinsed from the bird’s crown using 1 mL of tap water dispensed from a 1-mL pipette, guiding the flow of water down the bird’s bill and into the microcentrifuge tube. This process was repeated for all experimental flowers. The bird was then allowed to dry before testing it on the next experimental flower.

The microcentrifuge tubes containing pollen samples were spun at 60 Hz in a centrifuge for approximately 30 s. The supernatant was decanted until only a few drops containing the pollen pellet remained in the conical tip of the microcentrifuge tube. A drop of methylene blue dye was added to enhance the visibility of the pollen under the light microscope. To preserve the grains and prevent them from exploding or shrivelling, 95% ethanol was added to each sample to bring the final volume to 0.5 mL. To count pollen grains, samples were first vortexed for 15 s to resuspend the pollen grains. Then, four 5-μL drops were removed from each tube and placed separately on a hemocytometer for counting under a light microscope. The numbers of pollen grains in each drop were averaged, and the average was multiplied by 100 to yield estimates of the amount of pollen removed for each hummingbird visit.

Video analysis

Videotapes were analyzed using a VHS recorder (Panasonic model PV 4264) with a jog-shuttle feature that allowed analysis of still images, which were recorded at intervals of 1/60 s (= 60 frames per second; 1 frame = a composite of two different fields). For each hummingbird visit, we measured flying time, contact time, error time, bill entry angle, bill insertion time, and depth of maximum bill insertion. Handling time began when the bill or tongue first entered the flower opening and ended with the last image before the bill or tongue left the corolla. Contact time was the total time that some part of the bird’s body was in contact with the anthers. Error time was the time from when the bird’s bill or tongue missed the corolla opening to when the bill first entered the flower, following repositioning by the bird. Only errors that occurred before feeding were noted, and any probing of the flower after the first visit was disregarded.

We expected that the lower lip of flowers might enhance pollen removal either by altering the alignment (angle) of bill entry into the flower tube, by slowing bill insertion, or by altering the maximum depth of bill insertion. Accordingly, we measured entry angle, bill insertion time, and depth of maximum insertion for each flower visit. Bill entry angle was determined by laying an acetate sheet over the flower’s image on a video monitor and then drawing a horizontal line through the flower opening that bisected the flower into top and bottom halves. Using a protractor, entry angle was then measured as either the positive (top half) or negative (bottom half) deviation from the horizontal at exactly the point (= frame on videotape) at which the bird’s bill entered the corolla opening. Bill insertion time was measured from the time at which the bill tip or tongue entered the corolla opening until the time of maximum bill insertion. Depth of maximum insertion was measured at the time (video frame) of maximum bill insertion and equaled the distance from the junction of the upper and lower flower lips to the first dorsal feathers on the bird’s culmen. This measure provides an inverse function of maximum insertion depth by quantifying the amount of bill exposed at maximum insertion, i.e., larger values of maximum insertion depths indicate less bill insertion.

Data analyses

Because feeding behaviour of hummingbirds is often related to bill length (see Temes and Roberts 1993; Temes 1996), we used paired *t* tests to compare differences in handling times, contact times, bill insertion depths, bill insertion times, and pollen removal between lipmed and lipless flower states for each bird (*n* = 10 birds). These comparisons were performed on the means of each variable for each bird at each flower state, and all comparisons were two-tailed tests (MINITAB, Inc. 1989). Regressions were performed using the general linear models procedure in SAS (SAS Institute Inc. 1988) to examine the relationship between handling and anther contact times for lipmed and lipless flowers.

Results

Birds missed flower openings about five times as often at lipless than at lipmed flowers, and this difference translated into a fivefold increase in error times at lipless than at lipmed flowers (Table 1, rows 1 and 2). Once their bills had entered
Table 1. Paired t tests comparing feeding and anther contact variables (means of the means for each of 10 birds visiting 10 flowers of each type ± SE) between unmanipulated (lipped) flowers of Monarda didyma and flowers whose lips had been experimentally removed (lipless).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Lipped</th>
<th>Lipless</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of errors</td>
<td>0.86±0.18</td>
<td>4.83±0.98</td>
<td>4.07</td>
<td>0.0028</td>
</tr>
<tr>
<td>Error time (s)</td>
<td>0.03±0.01</td>
<td>0.17±0.20</td>
<td>4.35</td>
<td>0.0002</td>
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<td>Handling time (s)</td>
<td>1.74±0.63</td>
<td>1.51±0.26</td>
<td>2.10</td>
<td>0.01</td>
</tr>
<tr>
<td>Bill entry angle</td>
<td>13.39±3.17</td>
<td>12.61±2.99</td>
<td>0.40</td>
<td>0.7051</td>
</tr>
<tr>
<td>Bill insertion time (s)</td>
<td>1.01±0.07</td>
<td>0.69±0.06</td>
<td>5.59</td>
<td>0.0003</td>
</tr>
<tr>
<td>Maximum insertion depth (cm)</td>
<td>0.34±0.11</td>
<td>0.27±0.09</td>
<td>0.95</td>
<td>0.3361</td>
</tr>
<tr>
<td>Anther contact time (s)</td>
<td>0.51±0.08</td>
<td>0.23±0.04</td>
<td>3.63</td>
<td>0.0068</td>
</tr>
<tr>
<td>Pollen removed</td>
<td>91.05±13.33</td>
<td>58.00±8.78</td>
<td>2.55</td>
<td>0.032</td>
</tr>
</tbody>
</table>

*Note: All comparisons are two-tailed tests. See Materials and methods for definitions of variables.

*Of 10 visits per bird, mean of 10 birds.

1Degrees up from horizontal.

2Number of grains in the 0.5-mL sample.

flowers, however, handling times of hummingbirds were approximately 0.25 s longer at lipped than at lipless flowers (Table 1, row 3). Mean angles of bill entry did not differ between lipped and lipless flowers (Table 1, row 4), indicating that longer handling times at lipped flowers were not a consequence of birds being misaligned with the nectar source (a similar result was obtained when the analysis was conducted on the variances of angles of entry, results not shown). In contrast, birds reached their maximum depth of bill insertion nearly 0.33 s faster at lipless than at lipped flowers (Table 1, row 5). As a result, birds spent a greater proportion of their visits to lipped flowers than to lipless flowers in the act of bill insertion. Birds entered lipped and lipless flowers to the same maximum depths, however (Table 1, row 6).

Birds spent nearly twice as much time in contact with the anthers of lipped flowers than with the anthers of lipless flowers (Table 1, row 7). Anther contact times were significantly, albeit weakly, related to handling times at lipped flowers, but not at lipless flowers, suggesting that increased anther contact at lipped flowers may in part be a consequence of longer handling times at these flowers (lipped: $r^2 = 0.101, P = 0.0054, n = 75$; lipless: $r^2 = 0.0035, P = 0.616, n = 74$).

The amount of pollen removed by birds differed significantly between flower states (Table 1, row 8). Lipped flowers transferred nearly twice as much pollen to hummingbirds than lipless flowers.

Discussion

Although lips affect pollinator behaviour by reducing the frequency of insertion errors, our results suggest an advantage resulting from prolonged contact with a flower’s anthers and enhanced pollen removal. Differences between lipped and lipless flowers in duration of anther contact and pollen removal most likely were a result of differences in bill insertion times at lipped and lipless flowers, because we detected no differences in angles of entry or maximum bill insertion depths between flower treatments. An alternative explanation for our findings is that severing the lower lip of a M. didyma flower somehow altered the flower’s mechanical pollination system, rather than the bird’s behaviour, thereby lowering anther contact. We observed no mechanical differences between lipped and lipless flowers, although such differences may be subtle, especially if they occur within the floral tube, out of view of the observer. On the other hand, the videotapes indicated that, upon reaching maximum bill insertion, birds’ heads often had passed beyond the anthers such that no contact between anther and bird was occurring. Because birds entered lipped flowers more slowly than lipless ones, they were thus positioned beneath the anthers of a flower for a longer duration and had longer durations of anther contact as a result. Functionally, then, the lower lip of a M. didyma flower can be viewed as a device that affects bird behaviour and maintains the bird in the appropriate position for pollen removal for a longer period of time. Why the lip slows down the rate of bill insertion is unclear, but possibly the lip acts as a “crash barrier” that causes the bird to decrease its rate of entry to avoid collision. In that regard, birds occasionally were observed to hit the lower lip with their throats. Experiments using both real and artificial flowers having lips of various sizes and oriented at different angles might prove useful for evaluating the role of the lip as a “crash barrier” and also for evaluating the relationship between lip and anther architecture relative to pollen removal.

Whether the nearly twofold difference in pollen removal between lipped and lipless flowers translates into higher male reproductive success for lipped flowers requires a more detailed study of the links between pollen removal by birds and subsequent pollen deposition onto stigmas and resulting seed set (Snow 1989; Harder and Thomson 1989). Moreover, our study focused on the role of lips for male reproductive success; whether lips affect female reproductive success (i.e., pollen deposition on stigmas and seed set) of M. didyma flowers also needs to be determined. Our inspections of M. didyma flowers indicate that, when the flowers enter female phase, the anthers curl up over the upper lip of the flower, whereas the mature stigma curls down, partially blocking the flower opening. Such a stigma position in front of the flower opening results in a duration of contact between hummingbird and stigma that is twice as long as that between hummingbird and anthers (Rankin 1996) and may lessen any advantage lips may play in increasing the duration of stigma contact. If such differences in anther-stigma contact are present between male- and female-phase flowers of M. didyma, they would raise the intriguing possibility that
the lower lip of this flower functions primarily to enhance male reproductive success (see Bell 1985 for related discussion).

Conclusions: why do some hummingbird flowers have lower lips?

Our results indicate that the lower lip of *M. didyma* flowers enhances pollen removal by hummingbirds. This result does not mean, however, that selection by hummingbirds caused the origin of lower lips in *M. didyma* flowers. Most likely, *M. didyma*’s hummingbird-syndrome flowers evolved from some ancestor’s bee-syndrome flowers (Scora 1967). The lower lip was retained (perhaps with some modification in that it became more reflexed), so our results should be used to explain only the maintenance of the lower lip.

The next question is whether the presence of lips positively or negatively influence the fitness of hummingbird-pollinated plants in the context of hummingbird visitation, given the long-standing view that specialization on hummingbirds favors the loss of such structures (Grant and Grant 1968; Faegri and van der Pijl 1979). Our results here and elsewhere suggest that flower lips may enhance male fitness of hummingbird-pollinated plants via their effect on hummingbird behavior. Lips of hummingbird flowers act as a guide to reduce insertion errors by hummingbirds and to increase pollen removal by maintaining the hummingbird in an optimal location, directly under a flower’s anthers.

Flowers of *M. didyma* also are visited by bees, although infrequently (Whitten 1981). The degree to which lower lips are maintained on “hummingbird” flowers because they improve bee pollination, either by serving as a landing platform (e.g., Grant and Grant 1968) or by increasing floral attractiveness (e.g., Bell 1985), needs to be studied. For *M. didyma*, the lower lip is a preadaptation for hummingbird pollination, and selection pressures in the form of hummingbird visitors apparently would not favor the reduction or loss of the lower lip. Whether this interpretation applies to other hummingbird-pollinated flowers requires further experimental studies, ideally framed in a phylogenetic context.

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