

Floral isolation between ornithophilous and sphingophilous species of *Ipomopsis* and *Aquilegia*

(mechanical isolation/hummingbird pollination/hawkmoth pollination/coadaptation/*Ipomopsis aggregata*)

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ABSTRACT The *Ipomopsis aggregata* group (Polemoniaceae) and *Aquilegia formosa*–*Aquilegia caerulea* group (Ranunculaceae) in western North America contain species with ornithophilous flowers and related species with sphingophilous flowers. The ornithophilous and sphingophilous species are sympatric over large areas and remain distinct in some sites where they grow close together. Floral isolation—a combination of mechanical and ethological isolation—plays a significant role in the reproductive isolation. The primary pollinators of the ornithophilous taxa are the common western American species of hummingbirds, and the primary pollinators of the sphingophilous taxa are western hawkmoths. The ornithophilous and sphingophilous flowers are adapted to their respective primary pollinators. A corollary of these specializations is that the differences between the two types of floral mechanisms significantly reduce interspecific pollination. The floral isolation is incomplete. However, it acts not alone but in conjunction with ecological and seasonal isolation, which are also incomplete. The combination of these three incomplete external isolating mechanisms is sufficient to maintain the distinctness of the ornithophilous and sphingophilous species in some areas of sympatric coexistence.

Floral isolation is external reproductive isolation at the stage of pollination in angiosperms. It has two components: mechanical and ethological isolation. Mechanical isolation occurs when two (or more) plant species have different flower structures that reduce or prevent interspecific cross-pollination. The flowers of two species may be adapted for the same pollinator but deposit pollen on different parts of the pollinator's body. Or the contrasting species may have flowers adapted for different types of pollinating animals with different body forms. We are concerned with the latter type of mechanical isolation in this paper.

Ethological isolation frequently develops as a side effect of mechanical isolation involving different classes of pollinators. The structural differences that promote mechanical isolation often make the floral reward of one species more or less inaccessible to the normal pollinator of the other species. The pollinators recognize these structural differences and adjust their behavior so as to ignore the "wrong" species during foraging. Ethological isolation then accompanies and reinforces the mechanical isolation.

Because mechanical and ethological isolation are often combined in actual cases, and because the two components cannot always be readily distinguished, it is convenient to refer to them collectively as floral isolation. Examples of floral isolation have been reported in a number of plant groups (1, 2), but additional examples are needed. Two cases—one not previously explored (in *Ipomopsis*) and the other revisited (*Aquilegia*)—are described in this paper.

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These cases involve floral isolation between hummingbird-pollinated species and hawkmoth-pollinated species.

MATERIALS

The two species groups with which we are concerned are the *Ipomopsis aggregata* group (Polemoniaceae) and *Aquilegia formosa*–*Aquilegia caerulea* group (Ranunculaceae). Both groups occur in mountainous regions of western North America. They have been extensively studied by various botanists, including myself, from the standpoint of systematics, ecology, and pollination.

The plants are outcrossing diploid perennials with showy flowers. Most of the taxa in each group have either ornithophilous or sphingophilous flowers. The *I. aggregata* group has six ornithophilous and four sphingophilous taxa (3, 4); the *A. formosa*–*A. caerulea* group has seven ornithophilous and seven sphingophilous taxa (5, 6).

Most of the ornithophilous taxa in the *I. aggregata* group and all of those in the *A. formosa*–*A. caerulea* group have allopatric distribution areas. The sphingophilous taxa in each group are also allopatric. The allopatric taxa with a given pollination system are essentially geographical races belonging to the same species, and they are treated taxonomically as such in *Ipomopsis* (3) but not in *Aquilegia* (7). On the other hand, the ornithophilous species and the sphingophilous species are broadly sympatric in both *Ipomopsis* and *Aquilegia*. The species with contrasting pollination systems remain reproductively isolated in some sympatric localities but hybridize in other localities.

Internal sterility barriers are known to be weak in the *I. aggregata* group (8) and in the genus *Aquilegia* (9–11). Reproductive isolation between sympatric species is therefore determined mainly by external barriers.

We are concerned primarily with certain taxa in the two groups—namely, *I. aggregata formosissima* (ornithophilous), *Ipomopsis tenuituba* (sphingophilous), *A. formosa truncata* (ornithophilous), *Aquilegia pubescens* (sphingophilous), and *Aquilegia chrysantha* (sphingophilous). Furthermore, we are concerned only with the nonintrogressive forms of these taxa. However, the evidence is applicable and the conclusions can be extended to some other parts of the two species groups.

RESULTS

Ecological Isolation. The sphingophilous taxa of *Aquilegia* and of *Ipomopsis* (with one exception) occur in a higher elevational zone than do the ornithophilous taxa. The two classes of taxa live in different but contiguous plant communities. Thus in the Sierra Nevada, ornithophilous *A. formosa truncata* grows along streams, and sphingophilous *A. pubescens* grows in dry rocky places above the wooded streams. The two species occur within normal pollen dispersal range of one another without hybridizing in a number of known sites (ref. 12; unpublished data). Ornithophilous *I. aggregata*

and sphingophilous *I. tenuituba* have a parallel relationship in the Kaibab Plateau, Arizona, where the former occurs in montane forest and the latter in the neighboring subalpine zone (3, 8). Ornithophilous *I. aggregata collina* and sphingophilous *I. aggregata candida* occur in neighboring woodland and grassland habitats without hybridizing in various sites in Colorado (13). In these and other cases ecological isolation plays a leading role in the isolation of the ornithophilous and sphingophilous species (8, 12–16).

Since the sphingophilous taxa usually occur at higher elevations than their ornithophilous relatives, the former come into flower later. The flowering seasons of the two classes of taxa have different peaks but overlapping ranges. Some seasonal isolation thus exists, but it is incomplete (8, 12, 14).

Contrasting Pollination Systems. The primary pollinators of the ornithophilous taxa of the *I. aggregata* and *A. formosa*–*A. caerulea* groups are the hummingbird species with wide breeding ranges in western North America. *Selasphorus rufus*, *Stellula calliope*, and unidentified hummingbirds have been recorded on *I. aggregata formosissima* at four sites in the Sierra Nevada, California, and *Selasphorus rufus* and *Selasphorus platycercus* at three sites in northern Arizona (refs. 17 and 18; unpublished data). *Selasphorus rufus* and *Selasphorus platycercus* are also common visitors and pollinators of the related *I. aggregata aggregata* and *I. aggregata collina* in Colorado (17, 19–21). *Selasphorus rufus*, *Stellula calliope*, *Calypte anna*, and unidentified hummingbirds have been recorded on *A. formosa truncata* at seven sites in central and northern California (12, 14, 15, 22). In Colorado the related *Aquilegia elegantula* is pollinated by *Selasphorus platycercus* (16, 20).

The ornithophilous taxa of *Ipomopsis* and *Aquilegia* exhibit a suite of floral and inflorescence characters that correspond to the foraging habits of their hummingbird visitors. Such characters include flower color, orientation of flowers in the inflorescence, daytime nectar production, length and width of the floral tube, and pollination mechanism.

The primary pollinators of the sphingophilous taxa of the two plant groups are hawkmoths. *Hyles lineata*, the most common sphingid visitor, is a pollinator of *I. tenuituba* in Arizona (17, 18) and *I. aggregata candida* in Colorado (17, 21). It has also been found on *A. pubescens* in the Sierra Nevada, California (12, 14, 15), on *A. chrysantha* in Arizona (6, 14), and on the related *A. caerulea* in Colorado (16, 23). Species of *Sphinx*, *Eumorpha*, and *Manduca* also visit sphingophilous flowers of the two plant groups (6, 23, 24).

The flowers of the sphingophilous taxa of *Ipomopsis* and *Aquilegia* correspond to the characteristics and habits of hawkmoths in numerous ways: color, fragrance, orientation, vespertine and nocturnal nectar production, length and width of the floral tube, and pollination mechanism.

The length and width of the floral tube and the mouthparts in each pollination system illustrate the correspondence within a system and the difference between systems.

In the ornithophilous taxa the floral tube is trumpet-shaped and moderately long, being 16–24 mm long in *I. aggregata formosissima* (Fig. 1A) and 9–19 mm long in *A. formosa truncata* (Fig. 2A) (25). Such tubes correspond well with the dimensions of the mouthparts of western American hummingbirds. The average bill length of rufous hummingbirds together with a short tongue extension of 5 mm produces a mouthpart averaging 24 mm long in females and 22 mm long in males. The bill is 1.7 to nearly 2.0 mm wide in females, and it expands slightly in width during nectar uptake (25). In bill size *Selasphorus rufus* is representative of the group of widespread western hummingbird species, which includes also *Selasphorus platycercus*, *Selasphorus sasin*, *Calypte anna*, *Calypte costae*, *Stellula calliope*, and *Archilochus alexandri* (25).

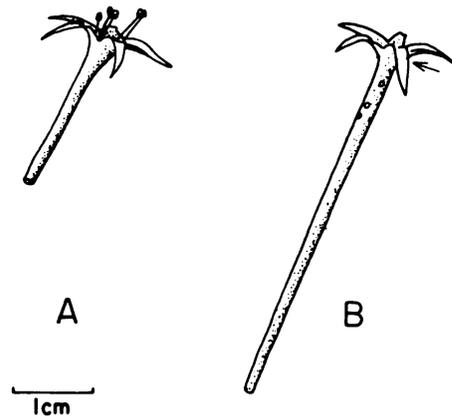


FIG. 1. Flowers of the *I. aggregata* group. (A) *I. aggregata formosissima*, ornithophilous. (B) *I. tenuituba latiloba*, sphingophilous. Circles indicate positions of included anthers; arrow indicates highest anther.

In the sphingophilous taxa, by contrast, the floral tube is very long and slender. The floral tube in *I. tenuituba* is 30–46 mm long (Fig. 1B); that in nonintrogressive *A. pubescens* is 28–39 mm long (Fig. 2B); and that in *A. chrysantha* is 39–69 mm long (Fig. 2C) (25). These floral tubes are well fitted for the long slender probosces of western American hawkmoths. The mean length of the proboscis in *H. lineata* is 38 mm; that of *Eumorpha achemon* is 43–50 mm; and the mean length ranges from 44 to 69 mm in different species of *Sphinx* (26). Probosces are even longer in *Manduca* (26).

It is fair to conclude that the ornithophilous flowers of *Ipomopsis* and *Aquilegia* and widespread western hummingbirds form a coadapted system (5, 25). Similarly, the sphingophilous flowers of these plant groups and western hawkmoths form another coadapted system (6, 17).

Effects of Differences Between the Ornithophilous and Sphingophilous Floral Mechanisms. The adaptive differences between the ornithophilous and sphingophilous flowers in the two plant groups are such as to inhibit interspecific visitations and pollen transfer by either hummingbirds or hawkmoths. The floral differences do this in various ways.

Consider first attraction. The sphingophilous flowers in the two plant groups are mostly pale yellow or white, sometimes light blue, and are visible at night. Nectar production takes place at dusk and night, and the flowers are fragrant. The ornithophilous flowers, on the other hand, are red and are highly visible by day but obscure at night. Nectar production in these flowers takes place during the daytime. The ornithophilous flowers are mostly odorless. Most hawkmoth feeding occurs in the dusk and night. Night-foraging hawkmoths can readily find and successfully feed on the sphingophilous flowers, but apparently they have difficulty locating the obscure and odorless ornithophilous flowers. Furthermore, the ornithophilous flowers normally offer no nectar reward for the hawkmoths at night.

Western American hummingbirds can reach the basal nectar in the shorter sphingophilous flowers of *Ipomopsis* and *Aquilegia*, but their nectar-extracting efficiency is much reduced here as compared with their foraging ability on ornithophilous flowers (25, 27). They cannot reach the basal nectar in the longer sphingophilous flowers of the two plant groups (25). When hummingbirds cannot forage successfully on a species of flower, they ignore those flowers. In several localities in Arizona the common widespread species of hummingbirds with medium long bills have been observed to ignore the long-tubed *A. chrysantha* (25). This is an example of ethological discrimination developing as a side effect of mechanical differences.

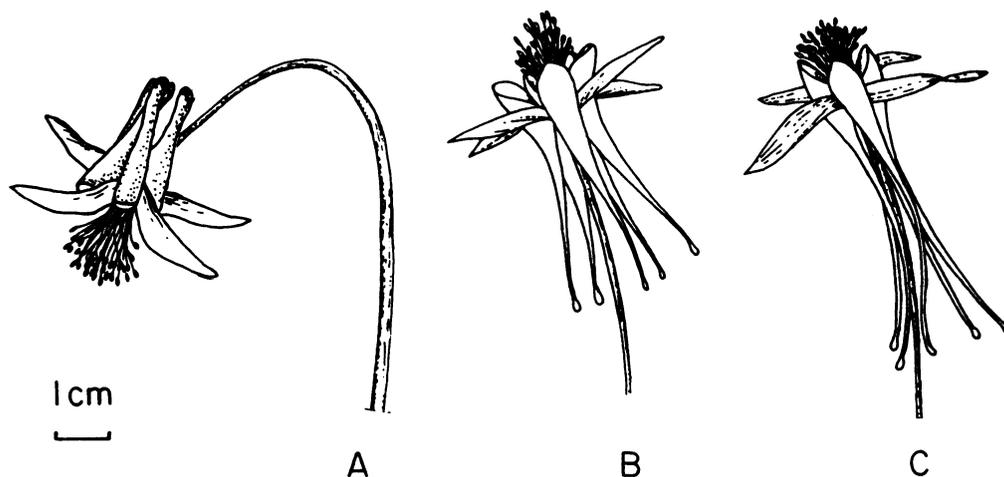


FIG. 2. Flowers of the *A. formosa*-*A. caerulea* group. (A) *A. formosa truncata*, ornithophilous. (B) *A. pubescens*, nonintrogressive form, sphingophilous. (C) *A. chrysantha*, sphingophilous.

Let us now consider day-flying hawkmoths. Floral tube length of ornithophilous flowers is not an obstacle for them; they can easily reach the nectar of such flowers. However, other features of the ornithophilous floral mechanism do present obstacles for hawkmoths.

Ornithophilous flowers of *Aquilegia* are nodding (Fig. 2A). The inverted floral spurs are readily accessible to hummingbirds hovering below and probing upward. Hawkmoths normally approach and probe flowers from above, and sphingophilous flowers are generally erect or ascending (Figs. 1B and 2B and C). Day-flying hawkmoths have difficulty in inserting the extended proboscis into nodding flowers of *A. formosa*. To be sure, a case of *H. lineata* probing spurs of *A. formosa* has been recorded (12), but this is a rare event in the overall behavior of *H. lineata*, which usually flies over *A. formosa* flowers without trying to visit them (ref. 14; unpublished data). This is an ethological component of floral isolation on the part of hawkmoths.

The pollination mechanism of many ornithophilous flowers of *Ipomopsis* and *Aquilegia* presents another obstacle to hawkmoth pollination. In many ornithophilous taxa of *I. aggregata* (Fig. 1A), for example, the anthers and stigma are exerted. Day-flying *H. lineata* feeds on *I. aggregata formosissima* in the Sierra Nevada. But owing to its long proboscis, the moth has to hover at a distance from the flowers, and neither the proboscis nor the body comes into contact with the anthers or stigma. Hawkmoths would also usually miss the stamens and styles of nodding ornithophilous *Aquilegia* flowers in those instances when they attempt to visit them.

The floral differences will reduce cross-pollination between ornithophilous and sphingophilous flowers by either hummingbirds or hawkmoths. They are not expected to block it completely, since both types of pollinators are flexible and opportunistic in their flower visits and could occasionally effect some interspecific pollinations. Furthermore, pollen-collecting bees are occasional visitors to both ornithophilous and sphingophilous flowers of *Ipomopsis* and *Aquilegia* (12, 14, 17). There is no mechanical isolation between the ornithophilous and sphingophilous species as far as such bees are concerned. The floral isolation is thus incomplete.

Behavior of Pollinators in Mixed Populations. In sites where ornithophilous and sphingophilous species are sympatric and yet remain distinct, the two forms occur in adjacent but different plant communities. Both ecological and floral isolation are operative, and it is difficult to distinguish their respective roles. Hybrid swarms containing individuals of both types of parental species, as well as various hybrid

products, present other complications, but at least they control or equalize the secular ecological factor. We can get some direct evidence concerning the floral isolation in such mixed populations.

Hybrid populations of red-flowered *I. aggregata formosissima* and white-flowered *I. tenuituba latiloba* occur in scattered localities in northern Arizona (8). One such hybrid population on Fern Mountain near Flagstaff has been studied with respect to pollinator behavior by Paige and Whitham (18). These workers originally considered it to be a polymorphic population of *I. aggregata* (18), but the population has been restudied and reidentified as a hybrid swarm of the parentage noted above (8).

The Fern Mountain population varies in flower color from red through shades of pink and in other characters. *Selasphorus rufus*, *Selasphorus platycercus*, and *H. lineata* forage on the population. There is preferential visitation of red-flowered plants by the rufous and broad-tailed hummingbirds by day, and of white-flowered plants by *H. lineata* after sunset. The birds also visit pink and white flowers to some extent, and the moths likewise visit pink and red flowers to some extent (18).

The percentage of the flowers visited by hummingbirds that set fruit was greater for red flowers (22%) than for white ones (11%), with medium pink flowers being intermediate (17%). Conversely, the percentage of the hawkmoth-visited flowers that set fruit was greater for white flowers (40%) than for red ones (23%), with medium pink flowers again intermediate (28%). Thus there are statistical differences in pollinator effectiveness. The birds are more effective as pollinators on the *I. aggregata* types, and the moths on the *I. tenuituba* types (18).

Populations containing *A. formosa truncata*, *A. pubescens*, and hybrids occur in the Sierra Nevada, California. A mixed population containing both parental species and their hybrids at Saddlebag Lake north of Yosemite National Park has been the focus of successive pollination studies (12, 14, 15). Rufous and calliope hummingbirds are the primary pollinators of *A. formosa truncata* in the general area away from the hybrid population; and *H. lineata* is the primary pollinator of nonintrogressive populations of *A. pubescens* in the general area. These three species are also the main flower visitors and pollinators in the Saddlebag Lake population.

The first study (14) reported preferential visitation by both hummingbirds and hawkmoths in the Saddlebag Lake population. The second study (12) called attention to some errors in the first study but underestimated preferential visitation in general. The third study (15) reconciled the differences and

stated the following conclusions based on all the available evidence.

Preferential visitation and pollination take place in the Saddlebag Lake population. Hawkmoths visit mainly *A. pubescens* and *pubescens*-like hybrid segregates. Hummingbirds are regular visitors of *A. formosa* and *formosa*-like hybrid segregates. Hummingbirds also visit *pubescens*-like hybrid segregates and introgressive *A. pubescens* either legitimately or illegitimately. In the illegitimate visits the birds obtain the nectar by piercing the spur tips with their bills while hovering outside the flowers and not contacting the stamens or styles, whereas the legitimate probes into the spurs bring about pollination. The preferential visitation and pollination are incomplete (15).

Long-tubed nonintrogressive *A. pubescens* seems to be a marginal nectar source for hummingbirds. Nonintrogressive *A. pubescens* in sites distant from a hybrid population is sometimes visited and sometimes ignored by hummingbirds (ref. 12; unpublished data).

A. elegantula and *A. caerulea* occur in neighboring habitats and sometimes in close proximity in the southern Rocky Mountains. In the area around Gothic, Colorado, the first species is pollinated mainly by broad-tailed hummingbirds and the second mainly by *H. lineata*. But pollen-collecting bumblebees visit both species. The floral isolation is thus incomplete. Hybrid plants occur sporadically, confirming the incompleteness of the isolation (16).

DISCUSSION

The *Ipomopsis* and *Aquilegia* examples shed light on the nature of floral isolation, its relation to ecological isolation, and ways in which it differs from other isolating mechanisms.

Most isolating mechanisms are more or less independent of one another, the strength of each mechanism is quantifiable, and the effects of the individual isolating mechanisms in combination are additive. Thus cross-incompatibility and hybrid sterility represent different sets of processes. They are independent in the sense that a plant group can have high crossability but low hybrid fertility or vice versa. The degree of crossability and hybrid fertility can be quantified. Internal isolation in the plant group is a product of both the compatibility and hybrid sterility barriers, acting additively.

Floral isolation in *Ipomopsis* and *Aquilegia* does not conform to these standards. Here floral isolation works in conjunction with ecological and seasonal isolation. Ecological isolation plays a leading role. Floral and seasonal isolation reinforce the ecological isolation. The three external isolating mechanisms are complementary in their action.

The ornithophilous and sphingophilous species live in different ecological communities which include different or partly different arrays of flower-visiting animals. If the communities remain distinct, even though they occur in neighboring sites, interspecific cross-pollination is uncommon. And if the habitats of the contrasting species remain discon-

tinuous, stabilizing selection weeds out the hybrid products that appear. The neighboring species normally reproduce within their respective communities. The set of external isolating mechanisms, including floral isolation as one component, keeps the reproduction within species limits.

Internal isolating mechanisms are relatively robust. Floral isolation and ecological isolation, by contrast, are fragile. They work effectively under stable environmental conditions but break down when environmental changes upset an existing equilibrium.

Floral isolation in the *I. aggregata* and *A. formosa*-*A. caerulea* groups is incomplete. This does not mean that it is unimportant as a species-separating mechanism. Floral isolation does not act alone; it operates in conjunction with other external isolating mechanisms which are also incomplete. Two or three incomplete isolating mechanisms acting jointly can bring about a high degree of reproductive isolation, as exemplified by cases of sympatric but distinct ornithophilous and sphingophilous species in *Ipomopsis* and *Aquilegia*.

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