
POLLINATOR SHIFTS AND THE ORIGIN AND LOSS OF PLANT SPECIES¹

Diane R. Campbell²

ABSTRACT

Pollinators have long been implicated in plant speciation. Peter Raven's earlier work was instrumental in integrating foraging energetics of animals into our understanding of how shifts in major pollinators influence the evolutionary diversification of floral traits. More recently, efforts by Raven and others in the area of conservation have inspired pollination biologists to consider the implications of pollinator shifts and losses due to human activities. This paper uses the shift between hummingbird and hawkmoth pollination as a model for exploring impacts of pollinator shifts on plant populations. Recent studies have quantified the degree of reproductive isolation due to such pollinators in several genera. Data from *Ipomopsis* Michx. further allow us to consider whether recent changes in pollinator regimes have demographic consequences for plant populations. A majority of plant populations may currently suffer from pollen limitations on seed production, but few data exist on the demographic consequences of poor reproduction. In *Ipomopsis*, reduced seed production due to pollen limitation can impact the number of individuals surviving to reproduce in the next generation. Some populations of *I. tenuituba* (Rydb.) V. E. Grant are estimated to have finite rates of increase less than unity, which can be explained in part by current low levels of hawkmoth pollination. In the absence of an increase in hawkmoths, selection for wider corolla tubes and other floral traits could, in principle, attract enough hummingbird pollination to result in a growing population, but models show that such evolution by natural selection may leave the population vulnerable to local extinction. We need more studies of the quantitative demographic consequences of changes in pollinator regimes. Such studies should consider how evolutionary changes influence the risk of extinction.

Key words: *Aquilegia*, coevolution, extinction, hawkmoth, hummingbird, *Ipomopsis*, *Nicotiana*, pollen limitation, pollinator shift, speciation.

Changes in pollinator regimes are thought to be of critical importance in speciation and diversification of the flowering plants. Such changes include qualitative shifts from one type of pollinator to another, quantitative shifts in the relative proportions of major types, and overall reductions in visits by animal pollinators that select for wind pollination or self-fertilization. In the past few decades, biologists have made considerable progress in testing the role of pollinator shifts in fundamental aspects of the origin of species: diversification of floral traits and development of reproductive barriers between incipient species. They have also begun to evaluate the role played by recent changes in pollinator regime for the potential loss of species. One major goal of this paper is to explore the roles played by pollinator changes in both the origin and the loss of species, using hummingbirds and hawkmoths as a case study and paying attention to the historical roots of these ideas, particularly those resulting from the work of Peter Raven and his colleagues. A second major goal is to integrate the two themes by considering how we can

incorporate evolutionary studies into examining the impact of pollinator losses. Where data are otherwise scarce in the current literature, I will focus on studies of *Ipomopsis* Michx. (Polemoniaceae), including ongoing work.

PETER RAVEN AND POLLINATION BIOLOGY

Pollination is central to so much of angiosperm evolution that it perhaps should not surprise us that so many diverse aspects of Raven's work have made a mark on pollination biology. Although Raven's experimental work in pollination focused mainly on specialized pollination systems in the Onagraceae, perhaps his widest impact on this field can be traced to three papers that introduced new conceptual approaches during the 1960s and 1970s. Prior to that time, much of pollination biology emphasized descriptive analysis of associations between flower characters and types of pollinators (Faegri & van der Pijl, 1966). One of the biggest changes in pollination biology occurred with a fuller incorpora-

¹ I thank Peter Hoch and Barbara Schaal for the invitation to participate in the 53rd Annual Systematics Symposium of the Missouri Botanical Garden. Comments by Mary Price, Robert Schlising, Nickolas Waser, and an anonymous reviewer contributed to revision of this manuscript. The author's work is supported by grant DEB-0542876 from the National Science Foundation.

² Department of Ecology and Evolutionary Biology, University of California, Irvine, California 92697, U.S.A. drcampbe@uci.edu.

doi: 10.3417/2007006

tion of the animal's perspective, a change precipitated, in part, by publication of Ehrlich and Raven's (1964) landmark paper on coevolution and Heinrich and Raven's (1972) fundamental paper on foraging energetics of pollinators. In addition, the idea that gene flow is too restricted to be responsible for holding together a species (Ehrlich & Raven, 1969) sparked a whole industry focused on estimating movement of pollen (e.g., Levin & Kerster, 1974). These three papers by Raven helped to launch a renaissance in pollination biology, as biologists increasingly recognized plant-pollinator systems as models for understanding major issues in ecological and evolutionary processes. As the ideas of gene flow through pollen, and of coevolution, are considered elsewhere in the symposium, I will focus on what follows on other influences, especially the perspective brought by considering animal foraging.

POLLINATOR SHIFTS, FLORAL DIVERSIFICATION, AND ORIGIN OF PLANT SPECIES

HUMMINGBIRD VERSUS INSECT POLLINATION

Some of the most compelling evidence for the role of pollinators in speciation comes from studies of related plant species that are pollinated either by hummingbirds or by insects. This emphasis is perhaps natural, given the longstanding interest in features often found in hummingbird-pollinated plants: red, tubular flowers that are relatively broad (compared, for example, to moth-pollinated flowers) and produce large quantities of relatively dilute nectar (compared to bee-pollinated flowers; Bolten & Feinsinger, 1978). Raven (1972) considered the question of why so many plants visited by hummingbirds have red flowers. He argued that birds learn to recognize red as a signal for a large caloric nectar reward even though they have no intrinsic preference for red, and that red is the signal because it is less conspicuous to most insects. This topic has continued to fascinate pollination ecologists and remains not fully resolved. Recent studies have altered the picture by demonstrating that bees can perceive red flowers, although the color is more difficult for them to distinguish against a green background of leafy vegetation (Chittka & Waser, 1997); this results in increased handling time and presumably renders red flowers less efficient, but not invisible, for bee foraging (Spaethe et al., 2001). The primary significance of reward energetics present in Raven's original argument for flower choice has been amply documented and reinforced. This emphasis on rewards was fleshed out in the highly influential paper by Heinrich and Raven (1972). The idea, simply put, was that the caloric reward of a flower could be used

to predict which pollinator species would visit it, the number of flowers visited per plant, and other aspects of foraging biology that could influence important features of plant reproduction such as the outcrossing rate. Along with the concept of coevolution between insects and plants elaborated by Ehrlich and Raven (1964), this paper stimulated a new generation to work on pollination from both the plant and animal points of view. In particular, it emphasized the idea that one could predict aspects of pollination by understanding the foraging energetics of the animals (Waddington, 1983), an idea that also owed much to the interjection of foraging theory (Pyke, 1984). Foraging energetics ultimately offered new perspectives on the adaptive significance of many plant traits, such as number of flowers per inflorescence (Wyatt, 1982), nectar distribution across flowers (Best & Bierzychudek, 1982), nectar guides (Waser & Price, 1983), and packaging of pollen (Harder & Wilson, 1994).

QUANTIFYING POLLINATOR-MEDIATED SELECTION AND REPRODUCTIVE ISOLATION

These ideas about foraging energetics also revitalized work on speciation resulting from pollinator shifts. Speciation involves divergence of phenotype and development of reproductive isolation, and both could be produced by pollinator shifts. Different types of pollinators (for example, bumblebees vs. birds) have different energetic requirements, morphologies, and sensory systems that may lead them to visit plants with different suites of floral traits. Such behavioral differences would produce divergent selection mediated by pollinators, potentially leading to differentiation of the floral phenotype, and could also generate reproductive isolation (see below). While this viewpoint was clearly articulated early on (Grant, 1949), it took much longer for biologists to document selection mediated by pollinators. In an introduction to an influential volume on pollination biology published two decades ago (Real, 1983), Raven remarked that it will ultimately be necessary to document that genetically variable floral traits actually contribute to reproductive success. This feat has since been accomplished for several species (e.g., *Raphanus raphanistrum* L. by Stanton et al., 1986; *Polemonium viscosum* Nutt. by Galen, 1996); however, most studies have focused on selection within a particular plant species. Only recently has the question of divergent selection and reproductive isolation by divergent pollinators been addressed with the same rigor. However, recent studies of genera with both hummingbird and hawkmoth pollination (*Aquilegia* L., *Ipomopsis*, and *Nicotiana* L.) offer repeated examples in which the extent of reproductive isolation mediated by pollinators has been quantified.

To see how reproductive isolation has been quantified, it is important to consider in more detail how pollinator behavior can generate it. With pollinator-mediated divergent selection, hybrids between the two plant species could have low pollination and, therefore, low fitness, a form of postzygotic reproductive isolation. Furthermore, if pollinators focused on a single species because of its more suitable reward or morphology, pollen movement between species would be restricted, causing prezygotic reproductive isolation as well. This contribution of behavior to reproductive isolation is referred to as ethological isolation ($RI_{\text{ethological}}$) and can be quantified as:

$$RI_{\text{ethological}} = 1 - \frac{\text{heterospecific plant transitions}}{\text{conspecific plant transitions}}$$

where transitions refer to movements made by pollinators in a setup with equal choices available. For cases of species visited predominantly by hummingbirds and hawkmoths, estimates for ethological isolation range from 0.50–1.00, a considerable range (Campbell & Aldridge, 2006) that begs further consideration. A second form of reproductive isolation (mechanical isolation) can also be produced by pollinators when they move pollen more efficiently between conspecific flowers because of differences in aspects of floral morphology such as the positions of reproductive organs.

CASE STUDIES WITH HUMMINGBIRDS AND HAWKMOTHS

Two species of *Aquilegia* illustrate the case of strong ethological isolation and show the contribution of at least one specific floral trait (Fulton & Hodges, 1999). *Aquilegia formosa* Fisch. has pendent, red and yellow flowers with short nectar spurs, whereas *A. pubescens* Coville has upright, pale flowers with long spurs. Although visited primarily by hummingbirds and hawkmoths, respectively, the two species produce hybrid zones in the southern Sierra Nevada of California, a situation originally studied by Chase and Raven (1975). In an array of *A. pubescens* in which half of the flowers were tied to make the flowers pendent and, thus, given the trait of the other parental species, hawkmoths visited upright flowers more than 10 times as often as pendent flowers (Fulton & Hodges, 1999). Manipulation of this one trait produced strong preference for *A. pubescens*; however, other floral traits such as flower color are likely also involved, as the preference was still not as extreme as observed in arrays of the two parental species (Fulton & Hodges, 1999). Although flower orientation is clearly involved in strengthening the current reproductive isolation, it is not clear whether this trait was

also involved in the original speciation event, particularly without information on how each trait influences the energetics of nectar extraction by hawkmoths. Hodges and his collaborators are now studying the genetic basis of the floral traits that maintain reproductive isolation (Hodges et al., 2002).

Nicotiana alata Link & Otto and *N. forgetiana* Hort. ex Hemsley in Brazil provide a similar example in which prezygotic reproductive isolation is normally strong (Ippolito et al., 2004). In experimental plots with only plants of the two species, hummingbirds visited *N. forgetiana* exclusively, and hawkmoths strongly preferred *N. alata*, in agreement with their pollinator syndromes. However, the presence of some hybrids led to high interspecific pollen transfer, producing a “hybrid bridge” between the species (Leebens-Mack & Milligan, 1998). When plots contained the two species along with F_1 individuals, hummingbirds visited both species, so that their movements could have produced interspecific gene flow, and backcrossing between hybrids and the parental species was also possible. This finding supports the suggestion of Arnold (1997) that even if F_1 hybrids rarely arise due to prezygotic ethological isolation, their presence can accelerate further gene flow between species.

RELATIVE IMPORTANCE OF SELECTION MEDIATED BY POLLINATORS AND HABITATS

Besides pollinator differences, an alternative form of selection that could drive plant speciation is differences in habitat such as soil types, other physical attributes of the environment, or biotic interactions that are unrelated to pollinators (Waser & Campbell, 2004). Adaptation to different habitats could generate postzygotic reproductive isolation if hybrids are unfit as a result of genomic incompatibilities or specifically unfit in the parental environments (Hatfield & Schluter, 1999). Chase and Raven (1975) proposed that habitat differences might actually be more important than pollinator differences in maintaining the species difference between *Aquilegia*. In principle, these two forms of selection could be distinguished by comparing the extent to which hybrids suffer from low pollination success versus low survival or other aspects of fitness. Few studies have examined pollination success of plant hybrids, although visitation to hybrids was studied in the *Nicotiana* arrays mentioned above (Ippolito et al., 2004).

In *Ipomopsis* (Polemoniaceae), the importance of both pollinator-mediated and habitat-mediated selection has been investigated. As in the two cases mentioned above, the two species involved differ conspicuously in floral traits. *Ipomopsis aggregata*

(Pursh) V. E. Grant has broad-tubed red flowers with high nectar production and often exerted anthers and stigma, whereas *I. tenuituba* has narrow-tubed, long, white or pale pink flowers with lower nectar production and strongly inserted reproductive parts. These two species are close relatives (Wolf et al., 1993), and Grant and Grant (1965) suggested they provide an example of recent or incipient speciation via pollinator-mediated divergent selection in which *I. aggregata* is pollinated by hummingbirds and *I. tenuituba* by hawkmoths. However, at some contact sites, the two species are visited by both pollinator types when present, and, as a result, they often form extensive hybrid zones in nature (Grant & Wilken, 1988; Wu & Campbell, 2005).

One such hybrid zone occurs in Poverty Gulch in Gunnison County, Colorado. Hybrids between the two species of *Ipomopsis* taken from that area are easily made, with just as many seeds resulting from heterospecific crosses as from conspecific crosses (Campbell & Waser, 2001). To determine how hybrids compare with the parental species in pollination success versus other aspects of fitness, Campbell and Waser (2007) planted more than 3000 seeds from conspecific and heterospecific crosses (F_1 reciprocal hybrids) into the two parental sites and a site where natural hybrids are common and followed the progeny for 12 years until nearly all of these monocarpic perennial plants had completed their life cycle. Differences in survival and reproduction both made substantial contributions to the fitness differences. Overall, survival of F_1 hybrids was equivalent to the average of the parental species; however, in the two parental sites, the direction of the cross was critical. Hybrids with *I. tenuituba* as the mother survived well only in the natural hybrid site, suggesting that habitat differences are important. One factor involved is water availability. The hybrid site is warmer and drier than the parental sites, and the hybrids have higher photosynthetic water-use efficiency than the parental species (Campbell et al., 2005). Pollination is also critical. In experimental arrays of potted plants with equal numbers of each of the parental species and hybrids, plants of *I. aggregata* received and donated the most pollen to stigmas of other plants (as estimated by dyes), thereby enjoying the overall highest pollination success (Campbell et al., 2002). Hybrid pollination success was intermediate between that of the two parental species in one experiment and equal to *I. aggregata* in another. In a separate study, hybrids produced and sired intermediate numbers of seeds to those of the parental species (Meléndez-Ackerman & Campbell, 1998). So, hybrids do not suffer low pollination success in situations where only hummingbirds are present.

These patterns in pollination success reflect primarily the visitation preference of hummingbirds for *Ipomopsis aggregata* and hybrids rather than *I. tenuituba* (Campbell et al., 1997). In this area, two species of hummingbirds, broad-tailed hummingbirds and rufous hummingbirds, make most of the flower visits. Their preference also results in partial ethological isolation, with about 7% as much pollen transferred between species as transferred conspecifically (Campbell et al., 2002). Mechanical isolation contributes as well, although to a lesser extent (Campbell et al., 1998).

The visitation preference of hummingbirds reflects the wider corolla tubes, red color, and greater nectar production of *Ipomopsis aggregata* (Campbell et al., 1997; Meléndez-Ackerman & Campbell, 1998). In particular, the preference of hummingbirds for *I. aggregata* generates directional selection for wide corolla tubes. One species of hawkmoth (*Hyles lineata*) is also a visitor at this study site but only in rare years (two years out of the last 20). In years when they are present, they disproportionately visit plants with narrower tubes. The combination of birds and moths in the natural hybrid zone then generates disruptive selection on corolla width, in line with the expectation that pollinator-mediated divergent selection maintains species differences (Campbell et al., 1997).

Campbell and Waser (2007) used life table analysis to show that differences in age-specific survival and fecundity both made large contributions to the patterns of lifetime fitness seen in the plants in the reciprocal transplant study described above. The low fitness of *Ipomopsis tenuituba* at *I. aggregata* sites could be explained by low pollination, whereas the low fitness of F_1 hybrids with *I. tenuituba* mothers at that site reflected poor survival. In summary, both pollinator-mediated and habitat-mediated selection contribute to the fitness differences seen in this hybrid zone and to maintenance of species differences. They may also have contributed to the original speciation between these sister species (Wolf et al., 1993).

Another way to approach the problem of the role played by pollinator-mediated selection is to compare cases where species hybridize with cases where they do not, to see if the lack of hybridization can be explained by lack of shared pollinators. Such a comparison is most informative if it involves the same species pair in different localities. *Ipomopsis aggregata* and *I. tenuituba* show considerable geographical variation in the extent of hybridization. At Grizzly Ridge on the north rim of the Black Canyon of Gunnison, Colorado, there are few if any morphological hybrids. That site thus provides an extreme contrast with the Poverty Gulch site, where hybridization is extensive. This difference can be explained

by differences in behavior of pollinators between the two sites. At Grizzly Ridge, the same species of hummingbirds and hawkmoths found at Poverty Gulch are completely species-specific in foraging. Experiments with potted plants in four combinations, representing all combinations of site of origin of the plants and site of observation, showed that the difference in ethological isolation was primarily the result of hawkmoths changing their behavior between sites (Aldridge & Campbell, 2007).

In summary, the extent of reproductive isolation due to behavior of pollinators has now been documented in at least a few cases, with *Aquilegia*, *Nicotiana*, and *Ipomopsis* serving as examples of species pairs involving hummingbird and hawkmoth pollination. More work will be needed, however, to quantify the general importance of these differences in pollinators versus differences in habitats, to the two facets of plant speciation: divergence in phenotype and development of reproductive isolation.

ANTHROPOGENIC CHANGES IN POLLINATOR REGIMES AND POTENTIAL FOR SPECIES LOSS

POTENTIAL CONSEQUENCES

Plants and their pollinators, like other parts of the natural world, face increasing peril from human activities. One example is the recently documented parallel decline in insect pollinators and insect-pollinated plants in Britain and The Netherlands (Biesmeijer et al., 2006). Understanding and mitigating these perils is an important challenge for pollination ecologists and an important arena for future work. Peter Raven's extensive work in conservation biology has helped to fuel wide concern over threats such as habitat loss and fragmentation, global warming, and invasive species (Raven, 2002). While a recent report from the National Academy of Sciences (National Research Council, 2007) points out the considerable gaps in our knowledge of effects on pollination, concern over the potential for harm is rising. Potential consequences of pollinator changes extend not only to native plant populations but also to agriculture, as 66% of the world's crops depend on honeybee pollination or pollination by other bees (Roubik, 1995).

Pollinator changes include shifts in species composition of pollinators and reductions or even losses of pollinators. These changes can potentially have demographic and/or evolutionary consequences for plant populations. Demographics can be impacted if (1) the change increases pollen limitation of seed production, and (2) the lowered seed production alters population growth. If the rate of increase for the population drops below replacement, the result could

even be local extinction, especially if seed production drops even farther in small populations (as in the Allee effect seen in *Banksia goodii* R. Br.; Lamont et al., 1993). Even if demographic changes are not seen, evolution could be altered if the new pollinator regime selects for different floral or reproductive traits.

POLLEN LIMITATION

Most effort to date has focused on direct demographic changes, rather than evolutionary ones. The first requirement for a demographic change has considerable evidence. In the 1970s, it was proposed that seed production would rarely be pollen limited (Charnov, 1979; Willson, 1979). These influential papers were based on application of current sexual selection theory to plants and contributed, along with the conceptual papers by Raven mentioned above, to a renaissance in pollination ecology. Among other things, the notion of sexual selection spawned a large number of studies of pollen limitation (Burd, 1994). Pollen limitation can be demonstrated by an increase in reproduction when pollen loads on stigmas are supplemented by hand pollination, ideally of all flowers on a plant to rule out compensatory changes due to re-allocation of resources between flowers. A recent meta-analysis of studies in 306 species across 80 plant families found that fruit production is limited by receipt of low levels of pollen in the majority of cases (Knight et al., 2005), contrary to the original prediction from sexual selection theory. Furthermore, populations that have recently suffered habitat fragmentation have lower levels of pollination, as well as lower reproductive success (Aguilar et al., 2006). What we don't know in these cases is how critical seed production is to the growth of the population, the second requirement for a demographic change. A population that increases its seed production may nevertheless fail to thrive if a density-dependent process, such as competition of seeds for a limited number of suitable germination sites, negates the higher seed production. Several studies have combined the extent of pollen limitation with population projection matrices (Knight et al., 2005) in a first attempt to look at the demographic consequences; however, in all of these cases, absence of density-dependence was assumed.

EXPERIMENTAL TESTS FOR DEMOGRAPHIC EFFECTS IN *IPOMOPSIS*

My colleagues and I have begun to examine the possibility of demographic effects of low pollination in *Ipomopsis aggregata* and *I. tenuituba* (Price et al., 2008). In populations of *I. aggregata* near Gothic, Colorado, hummingbirds are responsible for about

94% of all flower visits made by pollinators (Price et al., 2005). These populations are often pollen limited, with supplemental hand pollination of all flowers on a plant approximately doubling total seed production (Campbell, 1991; Campbell & Halama, 1993). We simulated this increase experimentally by adding the estimated number of seeds produced by five naturally pollinated plants or five plants with supplemental hand pollination (750 or 1500 seeds) to areas of 8 m². Seeds were added again the next year to simulate persistent pollen limitation, and each treatment was replicated six times. Doubling the seed input to simulate full pollination roughly doubled the number of seedlings that emerged in each 8-m² plot (means = 132 vs. 252 seedlings) and the number of individuals that survived to flower (7 vs. 17 flowering plants; Price et al., 2008). Over this range of densities corresponding to natural versus full pollination, per-capita success was independent of density even though density dependence was evident over a wider 10-fold range of seed inputs.

To estimate the demographic effect of full pollination, we calculated the finite rate of increase (λ) for a population using the observed values of age-specific survival and age at flowering, while estimating age-specific fecundity as the observed proportion of surviving individuals that reproduced, multiplied by the average number of seeds made by either fully or naturally pollinated plants. A population would have $\lambda = 1.14$ (positive growth) under a scenario of full pollination versus $\lambda = 0.94$ (negative growth) under a scenario of continued natural pollination (Price et al., 2008). In this case, low seed production due to low pollen transfer does have a demographic impact, and natural populations may currently be in some peril from low pollination.

POTENTIAL DEMOGRAPHIC EFFECTS OF POLLINATOR SHIFTS

Applying this approach to *Ipomopsis tenuituba* allows us to consider the potential demographic consequences of a shift from hummingbird to hawkmoth pollination. The reciprocal transplant studies at Poverty Gulch discussed above have indicated that *I. tenuituba* may be in decline even in its native habitat. In 1994, we planted seeds from 10 full-sib families into a natural population of this species. The 197 seeds we planted eventually produced five plants that survived to reproduce (or that were still alive at the time our estimate was made in 2005), and produced an estimated 172 seeds themselves (details given in Campbell & Waser, 2007), giving a pooled net reproductive rate of $R_o = 0.87$ and a pooled finite rate of increase of $\lambda = 0.98$. These values for R_o and λ are based on small samples,

but if taken at face value would lead *I. tenuituba* to be considered Vulnerable (VU) according to the IUCN Red List (IUCN, 2001). In addition, they were obtained in an experiment where seeds were planted just under the soil surface, which increases germination relative to seeds that are simply scattered as would occur naturally (M. Price, N. Waser, D. Campbell, A. Brody, unpublished data).

In part, the low success of *Ipomopsis tenuituba* can be attributed to low rates of hawkmoth visitation. Demographic studies indicate that the finite rate of increase for *Ipomopsis* is elastic to increases in fecundity (Campbell & Waser, 2007); a percentage increase or decrease in fecundity during the first big year of flowering (age five or six) can have a substantial effect on the finite rate of increase. Indirect evidence that the level of hawkmoth pollination influences fecundity comes from comparison of fruit sets in years with and without substantial numbers of hawkmoths. In the time span that we have studied this hybrid zone, hawkmoths were most abundant in 1993. Fruit set (% flowers that set fruit) that year in *I. tenuituba* populations was 90%, compared with 25% in 1992 and 26% in 2002, both years without hawkmoths (Campbell et al., 1997, unpublished data). Furthermore, in 1992, fruit set increased in clinal fashion from the high-elevation *I. tenuituba* populations to the lower-elevation *I. aggregata* populations where hummingbirds were more abundant, while no such cline was observed in 1993 (Campbell et al., 1997). These data suggest that high fruit set is obtained only in years when hawkmoths are present.

During the past 20 years, my colleagues and I have observed hawkmoths in substantial numbers in the surrounding area in only two years. It is possible that hawkmoth frequency was historically higher (Grant & Grant, 1965). The current rarity of hawkmoths in the higher mountains might conceivably be a recent anthropogenic change, perhaps from widespread use of insecticides in the desert areas, which harbor larger populations of these animals, and/or from other causes, although there are no data on population trends of moth species in the United States (National Research Council, 2007). Regardless of the reason for low abundance, it is useful to ask: would a higher abundance of hawkmoths maintain viable populations of *Ipomopsis tenuituba*? This question can be addressed by using a demographic transition matrix with elements corresponding to age-specific survival and fecundity, while also considering what happens when fecundity is multiplied by a factor that corresponds to frequency of years with hawkmoths. Assuming that fruit and seed set are linearly related to the frequency of years with hawkmoths, and fruit set is

k times as high when hawkmoths are present:

$$R_o = (1 - m)B + kmB$$

where m = frequency of years with moths and $B = R_o$ when only birds and no hawkmoths are present. In our case, $k = 0.90/0.25 = 3.6$. Noting that one out of 10 years in which plants bloomed in our experiment had hawkmoths ($m = 0.1$), and in that situation $R_o = 0.87$ seeds per seed planted, solving gives $B = 0.69$. Thus, we would expect $R_o = 0.69$ if hawkmoths were never present and $R_o = 2.49$ if hawkmoths were always present. It would take a minimum of two years out of 10 with hawkmoths to raise R_o above 1. This simplistic calculation ignores evolutionary change (see below) but suggests that these *I. tenuituba* populations are in some danger unless visitation by hawkmoths and/or hummingbirds increases. It is worth noting that this species combines a number of traits that could make it particularly vulnerable: self-incompatibility, a monocarpic life cycle with no seed bank, and some dependence on hawkmoths that are extremely variable from year to year. In North America, many species of butterflies and moths are regarded as threatened or endangered in various regions (National Research Council, 2007). While effects of such low pollinator numbers on plant populations are rarely studied, one exceptionally clear-cut case of endangerment due to low hawkmoth pollination is provided by the tree *Oxyanthus pyriformis* (Hochst.) Skeels in South Africa (Johnson et al., 2004).

We need many more such studies that combine pollination and demography in order to assess the severity of what some have referred to as a pollination crisis (Kearns et al., 1998). It is perhaps easier to list what we do know about the problem than what we don't know. However, from the plant side it is clear that little is known about demographic effects and about what types of plants will suffer the most. It is important to identify such situations in order to know where to focus conservation efforts, and this will likely be an area of much future work. Aguilar et al. (2006) found greater effect of habitat fragmentation on pollination for self-incompatible species, but the importance of most other traits has yet to be demonstrated.

AN INTEGRATIVE EVOLUTIONARY PERSPECTIVE ON THE CONSERVATION OF PLANT-POLLINATOR INTERACTIONS

PUTTING EVOLUTION INTO IMPACT ASSESSMENT

To date, most assessments of the impact of loss of pollinator have ignored the influence of evolutionary change on demographic processes. A few studies have considered the possibility that reduction in pollinators

would alter selfing frequency and inbreeding depression (e.g., Lazaro & Traveset, 2006); however, most other genetic or evolutionary changes have been ignored. It is now abundantly clear that some natural populations experience selection strong enough (Endler, 1986) to produce evolutionary change on short time scales (Reznick & Ghalambor, 2001). Adaptation of populations to new selective regimes could, in principle, happen quickly enough to prevent extinction, and the potential for this process has been demonstrated theoretically (Gomulkiewicz & Holt, 1995). Still, empirical data are lacking, and the potential for such evolutionary changes has hardly been considered in any particular case, especially outside of the case of global climate change (Rice & Emery, 2003). We need to consider the possibility of such evolutionary change in the context of loss of pollinators.

AN ILLUSTRATION BASED ON *IPOMOPSIS TENUITUBA*

In this section, I will outline how we might consider the possibility of evolution in response to pollinator loss using the case of *Ipomopsis tenuituba*. Large variation in fitness among the 10 full-sib families we planted suggests a high opportunity for selection on traits that influence reproduction (Campbell & Waser, 2007). If hawkmoths disappeared entirely from these populations, could these plants evolve characters making them more attractive to hummingbirds quickly enough that the risk of extinction would be ameliorated? Selection on traits such as corolla width is strong, raising the possibility that an increase in corolla width might increase hummingbird visitation sufficiently to raise reproduction above the replacement rate. Of course, this scenario would require that hummingbird abundances not decline below their present level, and there are some suggestions of recent declines in the hummingbird pollinators as well (Nabhan, 2004). Even if it were possible for an evolutionary change in traits to increase hummingbird visitation, this would lead to greater similarity between the two species, and, thus diversity in a phenotypic sense would not necessarily be maintained. Most of the data necessary to model the question are available, but sample sizes are, in some cases, rather small, so that the quantitative answers will be extremely rough. This example should be viewed mainly as a guide to the approach.

The first step is to estimate how fast a character, such as corolla width, would change evolutionarily due to selection. According to a simple quantitative genetic framework, the change in corolla width between generations can be predicted by the strength of phenotypic selection on the trait (measured by the

selection differential S) multiplied by heritability of the trait. A more precise prediction can be made by taking into account genetic correlations between characters (Lande & Arnold, 1983), but for simplicity, those correlations will be ignored here. This procedure is equivalent to assuming that the selection by pollinators is only on corolla width and no other genetically correlated characters. Campbell (1996) measured the heritability of corolla width under natural conditions in *Ipomopsis aggregata* as 0.30, indicating that 30% of the variation could be explained by the average effects of alleles passed on by parents. Combining that figure with the phenotypic selection exerted by hummingbird visitation when presented with arrays of both *Ipomopsis* species and their hybrids (selection differential = 0.064; Campbell et al., 1997) predicts an evolutionary change of 0.02 mm per generation, about 1/50 of the difference in this trait between the two species. This calculation assumes heritability would be identical in *I. tenuituba* (a simplistic assumption; see Roff & Mosseau, 1999) and ignores any selection arising from greater efficiency of hummingbirds at dispersing pollen from wider flowers (Campbell et al., 1998). Furthermore, it assumes directional rather than stabilizing selection on the character, in contrast with Gomulkiewicz and Holt (1995), who envisioned a shift from one optimal value to another under the new selective regime. Incorporating stabilizing selection instead can also be done using simple quantitative genetics models, but that approach was rejected here because the observed phenotypic selection was primarily directional (Campbell et al., 1997).

The second step is to allow fecundity to increase in the next generation to the extent predicted by how the new phenotype (in this case a corolla that is 0.02 mm wider) would increase pollinator visitation. These data are available from studies of hummingbirds visiting flowers in experimental arrays of the two species and hybrids (Campbell et al., 1997). The net reproductive rate would increase between generations as follows:

$$R_o = B[(v + bzt)/v] = B \left[1 + \frac{bz}{v}t \right]$$

where B = net reproductive rate in the original generation following loss of hawkmoths, v = current hummingbird visit rate for *Ipomopsis tenuituba*, b = slope representing the increase in visit rate per change in corolla width, z = the change in corolla width per generation, and t = time in generations. The part in square brackets expresses the proportional increase in visit rate relative to the original rate by hummingbirds. Notice that what matters is the strength of selection and the response to selection (z) relative to the original visit rate. Campbell et al.

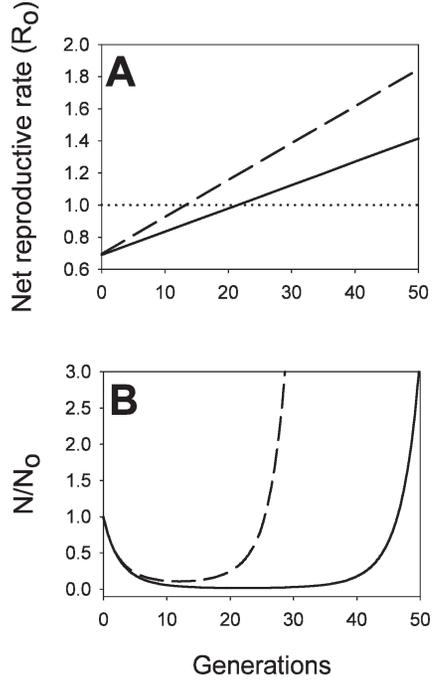


Figure 1. Estimated changes in the (A) net reproductive rate (R_o) and (B) population size (N) relative to its initial value (N_o), according to a model that allows for increased fecundity due to response to selection on a floral trait. Strength of selection (b) = 0.18 (solid lines) or b = 0.33 (dashed lines).

(1997) found an increase of 0.18 visits per millimeter from a mean of 0.17 visits per flower per hour for *I. tenuituba* plants (Campbell et al., 1997: fig. 3). Substituting,

$$R_o = 0.69 \left[1 + \frac{0.18(0.02)}{0.17}t \right] = 0.69[1 + 0.021t].$$

This approach assumes that fecundity is linearly related to visit rate. This assumption is unlikely to hold exactly, as seed production is expected to level off at high pollen receipt (Ashman et al., 2004), but may be a reasonable first approximation in this system since even *I. aggregata*, with its much higher visit rate, suffers pollen limitation.

The final step is to iterate the population size using this dynamic model for R_o . According to this simple model, it would take 22 generations for corolla width (198 years for this plant with its generation time of nine years) to increase sufficiently in response to selection for the net reproductive rate to rise above 1 (Fig. 1A). Using this change in R_o , in the meantime, the population size would decline to 2% of its initial value (Fig. 1B, solid line). Even using a more

optimistic value for how visit rate would increase ($b = 0.33$ based on a measure of selection taking into account correlated effects of other characters; Campbell et al., 1997), the population size would decline to 11% of its initial value before starting to recover after 13 generations (Fig. 1). Given that the current size of the population is probably in the hundreds, not thousands, these calculations suggest the population would likely be vulnerable to extinction through demographic or environmental stochasticity before it could evolve a sufficient increase in pollination level, despite the strong selection on corolla width.

All of the above considerations ignore the potential effects of hybridization. Hybridization can impact a population in one of two ways. If hybrids are relatively unfit, hybridization can lower the net reproductive rate of the population. Second, even if hybrids are fit, they may show signs of introgression, changing the population through genetic assimilation, as has occurred for example in *Helianthus* L. (Carney et al., 2000) and *Spartina* Schreb. (Anttila et al., 2000). In the case of *Ipomopsis* hybridization, hybrids with *I. tenuituba* as the mother and *I. aggregata* as the father had zero fitness in the home site for *I. tenuituba*, so hybridization would likely contribute further to decline of the population rather than to genetic assimilation.

I stress that these predictions concerning the effects of loss of hawkmoths, even if taken at face value despite the uncertainty in estimates of parameters, would apply only to the potential of local extinction from the region near Poverty Gulch, not global extinction of the species. Pollinator populations vary greatly in space, and hawkmoth numbers, in particular, seem to be highly variable. For example, in the columbine *Aquilegia caerulea* James, hawkmoths accounted for 68% of flower visitation in an Arizona population, while being absent entirely from another population in Colorado near our study site (Brunet & Sweet, 2006). In other populations of *Ipomopsis tenuituba* studied at Grizzly Ridge, hawkmoth visitation was 50% higher than at Poverty Gulch, which might be sufficient to maintain viable populations (Aldridge & Campbell, 2007).

CONCLUSIONS AND FUTURE DIRECTIONS

Since the publication of important conceptual papers by Raven in the 1970s, pollination ecologists have made much progress in understanding the evolution of floral diversity. In a number of cases, the influence of pollinators on microevolution of floral traits has been documented, and even quantified, through estimates of selection and heritabilities (Galen, 1996; Ashman & Majetic, 2006). Much recent

attention has been directed at the origin and maintenance of floral differences between species, and progress in this area will undoubtedly continue as new genetic tools and methods of analysis allow a finer look at the genetic basis of the traits (Bradshaw et al., 1998) and their fitness consequences (Morgan & Conner, 2001). We have come a long way in understanding how changes in pollination regimes can lead to diversification and origin of species. Much less, however, is known about how recent changes in pollination regimes impact the demographics of plant populations and might thereby lead to loss of plant species. Given the mounting evidence for decline of some pollinators, there is urgent need to design experiments that evaluate whether lower pollination will result in smaller populations of wild plants, particularly for those species that are already threatened. Such studies should consider not only direct demographic effects of lower pollination and seed production, but also the possibility of indirect demographic effects that occur through rapid evolutionary changes in trait values or in levels of inbreeding. It is time to heed Raven's (2002) call for action and bring the growing knowledge of ecology and evolution of pollination to bear on evaluating the impact of anthropogenic changes in populations of animal pollinators.

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