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# Associations between floral specialization and species diversity: cause, effect, or correlation?

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**Abstract** It has been proposed frequently, from Darwin's time onwards, that specialized pollination increases speciation rates and thus the diversity of plant species (i.e. clade species richness). We suggest here that the correlation between clade species richness and floral specialization is real, but that clade species richness is frequently the cause, not the result of floral specialization. We urge a broader, variance-partitioning perspective for assessing the causes of this correlation by suggesting four models of how the diversity-specialization correlation might come about: (1) floral specialization promotes initial reproductive isolation ("Initial-RI" model), (2) floral specialization promotes reinforcement of reproductive isolation upon secondary contact ("Reinforcement" model), (3) floral specialization reduces the extinction rate by promoting tighter species packing ("Extinction" model), (4) floral specialization is the result of high clade species richness, which increases the number of related species in communities, and thus selects for floral character displacement ("Character-Displacement" model). These hypotheses are evaluated by comparing the relationships between species richness, speciation mechanisms, and pollination precision, accuracy, and specialization in the broader literature and, more specifically, in four study systems: *Dalechampia* (Euphorbiaceae), *Collinsia* (Plantaginaceae), *Burmeistera* (Campanulaceae), and *Stylidium* (Stylidiaceae). These systems provide stronger support for the character-displacement hypothesis, wherein local species diversity drives the evolution of specialized pollination. Although the two reproductive-isolation hypotheses may hold for plants like orchids, with extremely precise pollination systems,

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the reproductive character-displacement hypothesis seems likely to be more important for plant groups with less precise pollination systems.

**Keywords** Character displacement · Pollination · Reinforcement · Reproductive isolation · Specialization · Speciation

## Introduction

Charles Darwin and dozens of evolutionary biologist since have argued that specialized biotic pollination was a key factor in the diversification and success of the angiosperms (Darwin 1876; Stebbins 1974; Regal 1977; Crepet 1984). In support of this idea, there is considerable evidence for a relationship between specialized pollination and high species diversity. For example, some groups like orchids have highly specialized pollination systems, apparently high speciation rates, and high species diversity (e.g., Dressler 1968; Cozzolino and Widmer 2005). Hodges and colleagues (Hodges and Arnold 1995; Hodges 1997) have suggested that the evolution of long nectar spurs, which promote more specialized pollination, was coincident with an increase in species richness. Additional statistical support comes from broad-scale studies by Ricklefs and Renner (1994), Dodd et al. (2000), Verdu (2002), and Jesson (2007) who showed that more species occur in lineages with specialized animal pollination than sister lineages with abiotic pollination (but see Bolmgren et al. 2003; Davies et al. 2004). Similarly, Sargent (2004) showed that lineages with bilaterally symmetrical flowers, which usually have more specialized relationships with pollinators, are more species rich (presumably because of higher speciation rates) than lineages with radially symmetrical flowers. Interestingly, a recent study with narrower taxonomic focus has shown that specialized pollination does not necessarily promote higher speciation rates (Smith et al. 2008), raising questions about the processes generating the relationship, if not the existence of the broad trend.

What mechanisms may have led to this apparent general association between pollination and species diversity? The most widely accepted hypothesis, expounded by Grant (1949, 1994), Stebbins (1970, 1974), and many others, holds that specialized pollination increases reproductive isolation, which in turn increases speciation rates (see review in Rieseberg and Willis 2007). However, there are other possible explanations. Here we outline four hypotheses or models that may account for the observed correlation. The goals of this paper are to provide a solid conceptual basis for evaluating these hypotheses, suggest possible sources of data for assessing the likelihood of the mechanistic models, provide circumstantial data for and against each model, and suggest how to proceed for obtaining more relevant data.

## Floral specialization

Before proceeding, we need first to discuss floral specialization. Generalized pollination is commonly defined as use of many of the available pollinators in a habitat and specialized pollination as use of few of the available pollinators (Waser et al. 1996; Fenster et al. 2004; Armbruster 2006). A related concept is phenotypic specialization, referring to morphological and other phenotypic traits of flowers that enforce restrictive pollination (Ollerton et al. 2007). Possible negative consequences of generalized pollination include loss of pollen (i.e. gene copies) from the reproductive pool and stigma blockage by heterospecific pollen.

We use here a more inclusive definition of floral specialization: any floral feature that increases the rate or efficiency of pollination by reducing gamete loss (see Armbruster et al 1999; Armbruster 2006). Floral specialization, in this broad sense, may evolve along one or more of at least four possible axes: (1) what animals are attracted (e.g. differences in rewards, reward accessibility), (2) when animals are attracted (season or time of day), (3) which visitors are pollinators, and (4) where pollen is placed on, and picked up from, pollinators. The first two axes relate to attraction of pollinators whereas the third and fourth relate to the fit between flower and pollinator. The latter two are affected by factors such as modular variance (Berg 1960, Armbruster et al. 1999), developmental repeatability (Diggle 1992, Pelabon et al. 2004), and floral accuracy, optimality, and precision in the pollination process (Armbruster et al. 2004, Hansen et al. 2006).

### *Divergence in attraction systems (rewards and advertisements)*

Attraction systems influence which animal species are attracted to the flowers. The nature of the pollinator reward can have major effects on the species attracted. For example, while nectar and pollen, the commonest rewards, attract a wide variety of invertebrates and vertebrates, floral oils, fragrances, and resins attract only certain species of bees. The animals attracted can also be affected by the nature of floral advertisements. These promote associative learning and increase the apparency of flowers, and include bright colours, distinctive shapes, and strong fragrances. Species attracted to a flower are also affected by reward accessibility. This is a commonly reported axis of specialization; for example, nectar is often secreted at the base of long tubular corollas or spurs (see Hodges and Arnold 1995; Hodges 1997; Muchhala 2006b). Specialization along this axis may result in a coevolutionary spiral or serial pollinator switches, as appears to be the case for the long spurs of some orchid and columbine flowers and their long-tongued pollinators (Darwin 1877; Nilsson et al. 1987; Nilsson 1988; Wasserthal 1997; Whittall and Hodges 2007; Ennos 2008; Hodges and Whittall 2008).

### *Divergence in timing of flower opening and receptivity*

Another axis of specialization is the evolution of a specific period when pollination can occur. Nearly all plants flower in a particular season. The shorter the period of flowering, the more specialized the pollination system, because fewer individuals, and often species, will be used as pollinators (e.g. Armbruster et al. 1992). A second form of temporal specialization is exhibited by plants whose flowers open for only part of the diel period. A common type of floral divergence along this axis is diurnal vs. nocturnal opening, which affects the species of potential pollinators (e.g. Muchhala 2003, 2006a). More restricted variation is seen when species differ in the time of day that flowers open and are receptive to pollination: e.g. morning-, afternoon-, or evening-opening flowers (Armbruster 1985, 1997, 2006; Stone and Wilmer 1998).

### *Divergence in morphological fit*

The morphological fit between the parts of flowers and flower visitors determines which floral visitors actually pollinate and at what efficiency. Specialization along this axis results in using only a subset of floral visitors as pollinators. This is seen in *Dalechampia* vines, which have either large or small bees as pollinators, but almost never both (Armbruster 1988, 1990), and in *Burmeistera* spp., which have bats as effective pollinators only when flowers are wide, and hummingbird as effective pollinators only when flowers are narrow (Muchhala 2007). Dressler (1968, 1981) has described similar patterns in orchids.

*Divergence in where pollen is placed on pollinators*

It has long been recognized that most flowers are either “nototribic”, placing (and picking up) pollen from the dorsal surface of the pollinator, or “sternotribic”, placing (and picking up) pollen from the ventral surface of the pollinator (Faegri and van der Pijl 1979; Keller and Armbruster 1989). Some flowers show further spatial specialization; e.g. most orchids, place pollen in precise locations on pollinators (Dressler 1968, 1981).

## Hypotheses

What processes may have led to the observed correlation between floral specialization and species diversity? In addition to the two hypotheses embedded in the “classic model” of reproductive isolation (1 and 2 below), we propose here two additional hypotheses. These four hypotheses (Fig. 1) include: (1) initial reproductive isolation (“Initial-RI” model), in which specialized plant-pollinator relationships increase speciation rates; (2) reinforcement of reproductive isolation (“Reinforcement” model), in which plants with specialized pollination experience more effective reproductive isolation on secondary contact, which in turn increases the rate of species accumulation; (3) reduced extinction rates (“Extinction” model) associated with floral specialization, and (4) reproductive character displacement (“Character-Displacement” model), in which speciation rates affect the number of species packed into communities, which in turn selects for floral divergence and specialization. Below we describe these hypotheses in further detail.

## Hypotheses 1 and 2: reproductive-isolation models

Pollinators may contribute to angiosperm diversity through their effects on reproductive isolation in two ways: either through shifts in pollinators generating reproductive isolation

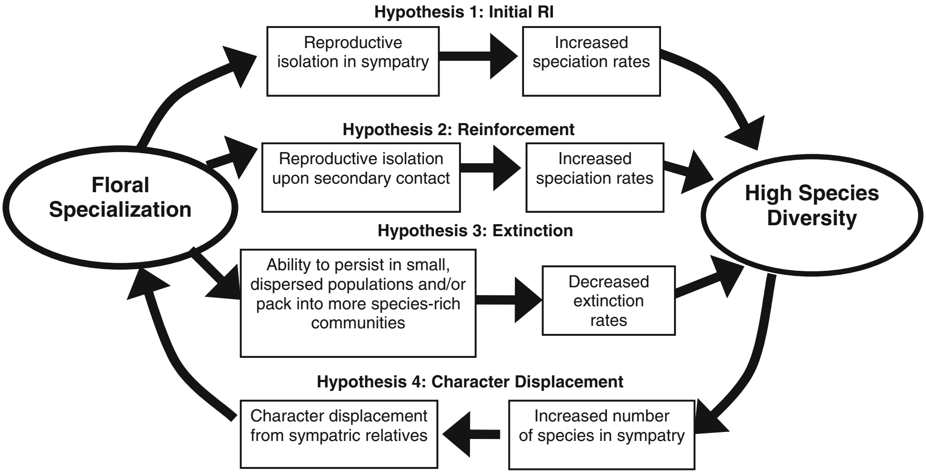

**Fig. 1** Four hypotheses explaining the correlation between floral specialization and the species richness of clades (phylogenetic groups)

and hence speciation (“Initial-RI model”), or through specialized pollination promoting the reinforcement of reproductive isolation upon secondary sympatry of incipient species (“Reinforcement model”; Grant 1949, 1971, 1994; Stebbins 1970, 1974; van der Niet et al. 2006; see Johnson 2007; Rieseberg and Willis 2007; Fig. 1).

Details of possible mechanisms of speciation through pollinator-induced reproductive isolation have been described (Schemske and Bradshaw 1999; Bradshaw and Schemske 2003). Neither these nor earlier studies, however, document whether pollen flow is sufficiently segregated to generate the degree of reproductive isolation needed for speciation (cf. above with counter-example described by Cooley et al. 2008).

A recent paper by van der Niet et al. (2006) provides evidence supporting the role of specialized pollination in the reinforcement of reproductive isolation. They suggest that shifts in pollination systems have contributed to speciation by reinforcement of reproductive isolation in Orchidaceae, Iridaceae, and Geraniaceae in South Africa. They showed that shifts in pollination between sister species were associated with edaphic differences and parapatry; this suggests that speciation was related to edaphic factors, but isolation was reinforced when and where they come together secondarily (see also Hopper 1979). In contrast, Moyle et al (2004) failed to find any genetic evidence for speciation via reinforcement in *Glycine* (Fabaceae), *Silene* (Caryophyllaceae), and *Streptanthus* (Brassicaceae). These divergent results may be resolved by the fact that, as van der Niet et al. (2006) pointed out, their data do not allow one to distinguish between pollinator divergence as the result of reinforcement (selection against hybridization) versus reproductive character displacement (selection against loss of gametes when gene flow is already precluded by postzygotic isolation; see hypothesis 4 below).

Reinforcement of reproductive isolation has been proposed repeatedly as an explanation for the evolution of pre-zygotic isolating in animals as well as plants (e.g., Dobzhansky 1937, Grant 1966). However, over the subsequent decades the concept has passed out of favour (Butlin 1989; Rice and Hostert 1993), and then back in (Noor 1999; Servedio and Noor 2003), based on theoretical issues. While much empirical data support the evolution of reproductive isolation predominantly as a by-product of divergence in allopatry or parapatry (Rice and Hostert 1993; McKinnon et al. 2004; Rieseberg et al. 2004; Orr 2005), some recent data support reinforcement as a mechanism of divergence (Coyne and Orr 1989; Hoskin et al. 2005; Lukhtanov et al. 2005). How this debate pans out in the future will either increase or decrease support for the Reinforcement model.

## Hypothesis 3: extinction model

Clade species richness is the difference between prior speciation and extinction; thus, extinction rates may also influence clade species richness. An association between specialization and clade species richness could be the result of floral specialization influencing extinction rates within a clade. Waser et al. (1996) used a simple model to show that plants with specialized pollination (using few pollinator species) might be expected to suffer higher extinction rates due to fluctuations in the population sizes of their special pollinators. This would lead to a negative relationship between specialization and species richness across lineages, which has never been reported (see Ollerton 1996).

Floral specialization along other axes (e.g. flowering time or pollen placement), however, may decrease extinction rates. This might occur if floral specialization allows more species to be packed into a community, because they do not suffer the negative effects of interspecific pollination (Armbruster et al. 1994; Armbruster 2006). Indeed, animal pollination generally, and any other floral adaptation that increases the effectiveness of intraspecific pollen flow, may allow plant populations to succeed at lower densities and when more widely dispersed than is otherwise possible (Raven 1977, Regal 1977). This might, in turn, affect reproductive success, population viability, species persistence, and ability to colonize new areas, leading to lower extinction rates and higher species richness.

## Hypothesis 4: character-displacement model

The species richness of clades often influences the number of related (e.g. congeneric) species that occur together in a community. Although very few data have been collected, it is self evident that species-rich genera should have, on average, more sympatric species that species-poor genera. Although there are certainly exceptions, as when related species are restricted to different parent material or microclimates, it is generally the case that groups with high species diversity have more species in the same community (see Kozak et al. 2005; Smith et al. 2007).

The co-occurrence of numerous sympatric species may generate selection for partitioning of the available pollinator fauna through floral adaptations along one or more of the axes of floral specialization. Such evolutionary change (character displacement) has been detected in many studies (e.g. Armbruster 1985; Armbruster et al. 1994; Fishman and Wyatt 1999; Muchhala and Potts 2007; Smith and Rausher 2007), and if this is a frequent response in sympatry, it may lead to a positive correlation between specialized pollination and clade species richness (Fig. 1). This hypothesis differs from the other three in positing that floral specialization is a result (rather than a cause) of species diversity.

Because the outcomes of floral character displacement and reinforcement of reproductive isolation are similar, they have often been treated as single process or otherwise variously conflated (e.g. van der Niet et al. 2006, Smith and Rausher 2007). However, in the context of speciation, the distinction between the two is of fundamental importance. When reinforcement of reproductive isolation occurs, it prevents gene flow that would otherwise cause two incipient species to become a single intermating population. In contrast, the Character-Displacement hypothesis relates to populations (species) that are already isolated (usually postzygotically) to a sufficient degree to prevent genetic amalgamation of the two populations.

The selective pressures involved in these two processes are very similar (loss of gene copies to unfit hybrids and/or unreceptive stigmas), as are many of the outcomes, such as floral character divergence and use of different pollinators. However, the two processes have very different effects on the relationship between pollination and speciation. Any shift in pollination system that increases the segregation of pollen flow (assortative mating) to a sufficient degree to prevent genetic fusion of populations is likely to increase the speciation rate, as described under hypothesis 1. In contrast, selection for improved mate discrimination between inter-sterile species will not affect speciation rates because reproductive isolation is already in place. Importantly, features that would not prevent genetic fusion of inter-fertile populations (because pollen segregation is incomplete) can be fixed by character displacement.

## Lines of evidence and study systems

Below we consider several lines of evidence that may help us evaluate the likelihoods of the four hypotheses described above. In addition to reviewing relevant literature, we present new data from four study systems with which we have had extensive experience: *Dalechampia* spp. (Euphorbiaceae), *Collinsia* spp. (Plantaginaceae), *Burmeistera* spp. (Campanulaceae), and *Stylidium* spp. (Stylidiaceae).

*Dalechampia* contains ca. 120 species of mostly perennial vines, distributed throughout most of the lowland tropics. The pollination unit is a blossom inflorescence (pseudanthium) comprising usually 10–15 staminate flowers, three pistillate flowers, and a gland that secretes resin in most species. These parts are subtended by two showy bracts. Pollination of most species is by resin-collecting bees, which use resin in nest construction (Armbruster 1993).

*Collinsia* and its close relative, *Tonella*, form a clade of about 24 annual species, primarily of temperate western North America. The pollination unit is the zygomorphic (bilateral) flower. Pollination is by long-tongued, nectar-feeding bees (which may also collect pollen; Armbruster et al. 2002).

*Burmeistera* is exclusively neotropical, with 102 species distributed from Guatemala to Peru. Flowers are protandrous and zygomorphic, with staminate and pistillate tissues partially fused into a column that first deposits pollen and then picks it up with the stigma. The genus is primarily bat-pollinated, with one known switch to hummingbird pollination (Muchhala 2003, 2006a).

*Stylidium* contains over 250 species of herbs, perennial rosette plants, and small shrubs, most of which are endemic to Australia. The pollination unit is zygomorphic flower, which characterised by the fusion of staminate and pistillate tissues into a motile, protandrous column. Pollination is by nectar-feeding bee flies (Bombyliidae) and small solitary bees (Armbruster et al. 1994).

*Dalechampia* blossoms have low floral integration, precision, and accuracy, compared to the other three genera (see Fig. 2; Armbruster et al. 2004). *Collinsia* has intermediate integration (fusion) and accuracy. *Burmeistera* and *Stylidium* flowers are highly integrated (fused), with greater precision and accuracy. The number of coexisting, co-flowering species is higher in *Burmeistera* and *Stylidium* than in *Dalechampia* and *Collinsia*, and the ability of co-occurring species to achieve segregated pollen flow follows a similar trend across genera. These taxa thus fit the predicted relationship between species diversity within communities and floral specialization. The above pattern suggests that the degree of floral specialization may also be influenced by the extent of floral integration and precision.

The remainder of this contribution attempts to assess the relative likelihoods of the four hypotheses described above. It is important to note that these hypotheses are not mutually exclusive, but instead possible mechanisms whose relative frequency we wish to assess. In the following sections, we will focus primarily on four lines of evidence: the degree of segregation of pollen flow provided by floral specialization, the extent of hybridization and types of isolating mechanisms, the nature of macroevolutionary shifts in pollination systems, and the relatedness of sympatric species (Table 1).

## Degree of segregation of pollen flow

Under the Initial-RI and Reinforcement models, which depend on prezygotic reproductive isolation, we expect pollination differences to segregate pollen flow very effectively (see Gegear and Burns 2007). Consideration of population-genetic models suggests that such assortative pollen flow must be nearly complete for divergence to occur in the absence of strong selection. The most appropriate population genetic model to compare against is the relationship between migration (gene flow) and random genetic drift: $F_{eq} = 1/(4\ Nm + 1)$,

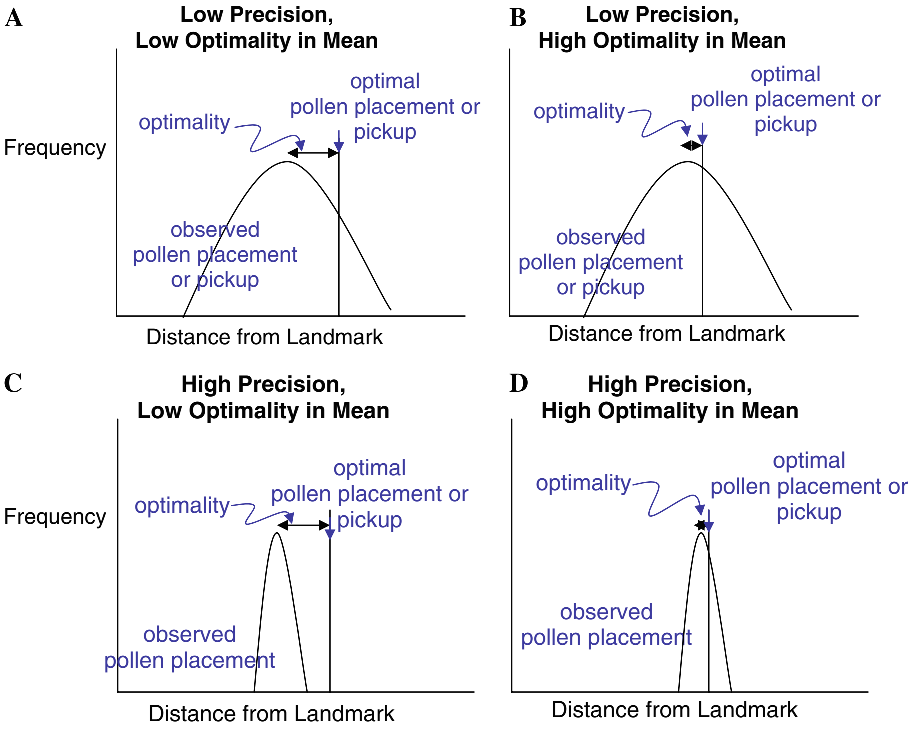

**Fig. 2** Adaptive accuracy, precision, and departure of the mean from the optimum, in the context of pollen placement and pollen pickup. **a**. With low precision (high variance in stigma/anther position) and low optimality (large deviation of mean stigma/anther position from the optimum). **b**. With low precision and high optimality (small deviation of the mean from optimum). **c**. With high precision (low variance in stigma/anther position) and low optimality. **d**. With high precision and high optimality. Adaptive accuracy is the joint effect of precision and optimality on fitness. More specifically, selection minimizes adaptive inaccuracy, which is the sum of the trait variance and the square of the departure of the trait mean from the optimum value (see Armbruster et al. 2004; Hansen et al. 2006). "Frequency" refers to frequency distribution of distances of stigmas or anthers from the relevant floral landmark (e.g. the site of the reward)

where $F_{eq}$ is equilibrium autozygosity (a measure of drift), N is the population size, m the fraction of the population migrating per generation. Note that Nm is thus the number of migrants per generation. When 4 Nm >1, the two populations are so strongly linked by gene flow that they function essentially as a single panmictic population (Roughgarden 1979). Hence, only a tiny amount of gene flow will prevent divergence of populations by genetic drift (Wright 1940, Roughgarden 1979). The net effect of selection and gene flow can be assessed by considering the magnitude of s, the local selection coefficient, in relation to m; s must exceed the value of m for selection to outweigh gene flow (Wright 1940). Thus, in general, weak selection will not result in divergence with moderate gene flow, although strong selection can do so (Wright 1940, Roughgarden 1979). We can conclude that the equivalent of one inter-"morph" pollination every other generation will swamp out differentiation under all but very strong selective pressures.

How frequently do pollinators actually provide such a high degree of segregation of pollen flow in nature? Barriers to gene flow may be manifested through sympatric congeners using different pollinator species (ethological isolation) or, alternatively, through sympatric congeners placing and picking up pollen accurately (and precisely) on different

**Table 1** Predictions of the four hypotheses for the relationship between clade species richness and pollination

| Lines of evidence | Hypotheses | | | |
|---|---|---|---|---|
| | (1) Initial-RI<br>Floral specialization increases speciation through initial reproductive isolation | (2) Reinforcement<br>Floral specialization increases speciation through reinforcement of reproductive isolation | (3) Extinction<br>Floral specialization decreases extinction by allowing persistence in smaller, dispersed populations | (4) Character-Displacement<br>High diversity increases specialization through character displacement of sympatric relatives |
| Degree of segregation of pollen flow | Floral specialization *prevents* interspecific pollen transfer | Floral specialization *prevents* interspecific pollen transfer | Floral specialization *reduces* interspecific pollen transfer | Floral specialization *reduces* interspecific pollen transfer |
| Hybridization and isolating mechanisms | Natural hybrids are moderately common, artificial hybrids are very *common* | Natural hybrids are moderately common, artificial hybrids are very *common* | Both natural and artificial hybrids are *rare* | Both natural and artificial hybrids are *rare* |
| Macroevolutionary shifts between pollination systems | Qualitative shifts without intermediate stage are common | Qualitative shifts without intermediate stage are common | No clear prediction | Quantitative shifts and qualitative shifts with intermediate phase predominate |
| Phylogenetic patterns of sympatry | Sister species are *often* sympatric | Sister species are *often* sympatric | Sister species are *rarely* sympatric | Sister species are *rarely* sympatric |

locations on shared pollinators (mechanical isolation; cf. Grant 1949, 1994). Below we consider each barrier in greater detail.

### *Do pollinators of sympatric congeners differ enough to generate reproductive isolation?*

According to Waser and colleagues (Waser et al. 1996, Waser 1998, 2001), ethological isolation is nearly always incomplete because most plants are visited by several to many species of pollinators, which also visit many kinds of plants (generalized pollination; see Waser et al. 1996). Hence, in this view, pollinator differences are usually statistical rather than absolute and *not* likely to generate reproductive isolation or promote speciation (see also Waser and Campbell 2004).

More focused studies provide ample empirical evidence that significant proportions of pollen grains commonly move between different floral types despite them having differences in pollinator faunas (e.g. Armbruster and Webster 1982, Armbruster and Herzig 1984). Other data illustrating the lack of complete isolation come from studies of rampant hybridization between species with large differences in what pollinates them most of the time (Aldridge and Campbell 2007; Hersh and Roy 2007), including studies of gene flow between highly specialized, sexually deceptive orchid species using different pollinator species (Cozzolino et al. 2005; Cozzolino and Scopece 2008).

Many orchids may be a notable exception to the general observation that differences in specialized pollination usually do not generate reproductive isolation. Most species have pollen grains united into pollinia, which may reduce the “random” component of pollen flow. In conjunction with this, orchid species often have very specialized relationships with pollinators, where a specific type of reward or mimicry system operates. Together, these features may permit closely related sympatric orchid species to have perfectly segregated pollen flow and reproductive isolation in the absence of post-zygotic isolating mechanisms (Dressler 1968, 1981; but see Cozzolino et al. 2005; Cozzolino and Scopece 2008, as noted above). Thus, use of different pollinators may maintain reproductive isolation between sympatric incipient species and thus increase the net speciation rates in orchids either through Initial-RI (see Johnson 2007) or Reinforcement (see van der Niet et al. 2006).

Outside of orchids, however, it seems likely that plant-pollinator interactions rarely generate sufficiently extreme segregation of pollinator species to effect complete reproductive isolation (Waser 1998, 2001; Waser and Campbell 2004). We must look elsewhere to account for the correlation between specialized pollination and species richness (speciation) that appears to hold in many plant groups.

### *Can differences in location of pollen placement effect reproductive isolation?*

Even if sympatric incipient species share pollinators, it may still be possible that they experience prezygotic isolation if they place pollen and pick it up from different locations on shared pollinators. This may occur if pollen placement and stigma contact with pollinators is generally very accurate and precise (see Fig. 2). The best example of highly accurate and precise pollination is again among members of the Orchidaceae, in which most species have pollen grains united into pollinia. Pollinium placement can be very precise and correspond exactly to the position of stigma contact. These features may permit closely related sympatric species to have perfectly segregated pollen flow and reproductive isolation when sharing pollinators and in the absence of post-zygotic isolating mechanisms

(Dodson 1962; Dressler 1968, 1981; but see Cozzolino et al. 2005). Thus, use of different locations on the same pollinator may maintain reproductive isolation between sympatric incipient species and thus increase the net speciation rates in orchids (see Johnson 2007) via either the Initial-RI or Reinforcement model (or both).

In most situations, however, such segregated pollen flow appears rare. In a study of assemblages of unrelated humming bird-pollinated plants, Murcia and Feinsinger (1996), for example, found large amounts of interspecific pollen transfer, despite large differences in floral architecture and pollen placement.

### *Comparison of study systems*

Our four study systems exhibit a strong gradient in floral integration, accuracy, precision, and potential to segregate pollen flow among sympatric species sharing pollinators. *Dalechampia* blossoms show the least integration due to the pseudanthial nature of the pollination unit. They also have the lowest accuracy and precision (see Fig. 2), and are unable to segregate pollen flow among sympatric species that use the same pollinators at the same time (Armbruster and Herzig 1984; Armbruster et al. 2004). Low accuracy and precision may be the result of conflicting selection for herkogamy (spatial separation of pollen and stigmas) because it reduces self pollination in this group of self-compatible plants (Fig. 2c).

*Collinsia* spp. has more integrated flowers and higher accuracy and precision (Armbruster et al. 2002). There appears to be some ability to segregate pollen flow among sympatric species that share pollinators, but not to a level that would prevent gene flow. As in *Dalechampia*, self-pollination is prevented or delayed by herkogamy, and this may select against greater optimality and precision (Armbruster et al. 2002, 2004).

*Burmeistera* flowers are highly integrated, with staminal and pistillate tissues partially fused into a column. Separation of sexual functions in time (protandry) rather than space combined with floral integration appears to have allowed response to selection for high optimality and precision. The development of the staminal tube is coordinated with the style such that the two function as a single unit (the "column"), initially releasing pollen from the tip and then receiving pollen onto the stigma in same location (Cronquist 1982; Muchhala 2003, 2006a, Muchhala and Potts 2007). Muchhala (2006a, 2008) showed that species of *Burmeistera* diverge from one another in the length of the column. Detailed flight cage experiments with pairs of *Burmeistera* species showed that the greater the difference in column length, the less pollen is transferred interspecifically (Muchhala and Potts 2007). Furthermore, column lengths of assemblages of co-occuring *Burmeistera* are significantly overdispersed relative to null expectations (Muchhala and Potts 2007). However, even for the species pair in the flight cage experiments with the greatest difference in column length (*B. borjensis* and *B. sodiroana*), more than 15% of the pollen was transferred between species. *Burmeistera* flowers in nature receive similar levels of heterospecific *Burmeistera* pollen (approximately 20% on average; Muchhala 2006a). Thus placing pollen in different locations on the pollinator appears to be unable to generate reproductive isolation even in this specialized system. Were this amount of gene flow to occur between incipient species in sympatry, the two populations would simply introgress back into one population.

In *Stylidium* the fusion of male and female tissues is very similar to that seen in *Burmeistera*, although the *Stylidium* column is motile and places and picks up pollen by springing forward from a recurved position. Thus, the flowers are highly accurate and precise, with the ability to partition pollen flow among sympatric species using the same pollinators. There is sufficient precision to enhance male fitness (getting pollen to the right stigmas), but as in *Burmeistera*, it seems unlikely that pollen flow is sufficiently segregated to preclude gene flow (Armbruster et al. 1994).

This analysis of four study systems reveals a range of integration, precision, and accuracy in pollination. Most plants fall out somewhere in the middle of this continuum; very few groups show the degree of integration and accuracy seen in *Burmeistera* and *Stylidium*. This suggests that most plants will not experience complete reproductive isolation even if they place pollen in different average locations on shared pollinators. These results do not support the Initial-RI and Reinforcement hypotheses, but instead bolster the Character Displacement and Extinction hypotheses (Table 1).

## Hybridization and isolating mechanisms

Because the Initial-RI and Reinforcement hypotheses assume prezygotic (ethological or mechanical) isolation, if they are correct we expect hybrids will be relatively easy to obtain in artificial crosses. We should also find the occasional hybrid in the field, because, in incipient cases, reproductive isolation may still be leaky, and it is hybridization that selects for secondary reinforcement. In contrast, the Character-Displacement and Extinction hypotheses assume post-zygotic isolation is already in place and therefore predict very little artificial or natural hybridization (Table 1).

To evaluate these ideas we wish to know whether species with specialized vs. generalized pollination systems are exclusively prezygotically or postzygotically isolated. Systematic data are hard to come by, however, although assessing the commonness of natural and artificial hybrids is a good start. Orchids are better studied than most groups. Here hybrids are commonly formed by artificial crossing, and hybrids are occasionally reported in the field (Dressler 1981), indicating that many species are exclusively prezygotically isolated.

The best data on orchids come from a recent comparison of food-deceptive and sexually deceptive orchids (Scopece et al. 2007). Food-deceptive orchids have strong post-mating and weak pre-mating isolation barriers, while sexually deceptive orchids have the opposite. Sexual deception reflects unusual specialization based on chemical exploitation of one or a few pollinators species, whereas pollination in food-deceptive orchids is more akin to pollination of “normal” flowers. Interestingly, post-mating isolation has evolved in a clock-like manner, whereas pre-mating barriers have not, supporting the idea that, for angiosperms with “normal” pollination systems, speciation commonly occurs through gradual divergence in allopatry. Hybridization and introgression is more common between sympatric sexually deceptive orchids than food-deceptive orchids (Cozzolino et al. 2005; Cozzolino and Scopece 2008).

In our four study systems, hybrids are seen very infrequently in the field, and have almost never been produced in the greenhouse. All the data indicate that isolation among related species is postzygotic. The clearest data come from greenhouse experiments with *Dalechampia*. Hybrids between *Dalechampia* species are almost never found in the field, even when pollinators move between closely related species (e.g. Armbruster and Steiner 1992). An extensive interspecific crossing program between both sympatric and allopatric species in the greenhouse at the University of California Davis, conducted 1979–1981, resulted in no definitive hybrids (Armbruster and Herzig 1984; Armbruster “unpublished data”). This suggests strongly that there are effective postzygotic barriers to hybridization.

A crossing program between populations of the *D. scandens* species complex in the NTNU Biology greenhouse at Trondheim, Norway, showed surprisingly strong postzygotic barriers between putatively conspecific populations that showed small morphological differences (Pelabon et al. 2005). Thus lack of hybrids in the field and greenhouse indicate strong postzygotic reproductive isolation. This, combined with the lack of evidence for prezygotic isolation, suggests that floral specialization and divergence in sympatry have not been involved in speciation.

Several years of collecting *Burmeistera* in sites throughout Ecuador similarly revealed very few suspected natural hybrids (Muchhala, "personal observation"). Interspecific crosses of sympatric species never resulted in seed set, although sample sizes were small (Muchhala, "unpublished data"). A study with a larger sample size provides even stronger evidence for post-zygotic rather than pre-zygotic isolation barriers. From Feb. to Mar. of 2004, crosses were made between two populations of *Burmeistera sodiroana*, one from Bellavista Reserve (BV) on the western slopes of the Andes of Ecuador and one from Yanayacu Reserve (YC) on the eastern slopes. These populations differ in the length of the staminal column, but are otherwise morphologically identical (Muchhala 2006a), and conspecific according to a phylogenetic analysis (Knox et al. 2008). This experiment was planned to verify that the populations could be crossed, in order to then be able to estimate the amount of pre-zygotic reproductive isolation that the difference in column length would provide should the populations come into secondary contact (cf. the Reinforcement hypothesis). However, results demonstrated that post-zygotic barriers already existed between the populations. Flowers from BV crossed with BV pollen donors set 1767 seeds (±356.0 SE) per cross on average while those crossed with YC pollen donors only set 1028 (±194.5). Similarly, YC flowers crossed with YC pollen donors set 972 seeds (±224.2) while those crossed with BV pollen donors only set 231 (±156.6). Decreases in the between-population crosses were due to a combination of increased fruit abortions and less seeds per fruit (Muchhala, "unpublished results"). These results suggest that post-zygotic barriers evolve before differences in column length, and specialization is more likely a result of character displacement rather than a cause of reproductive isolation or reinforcement.

A similar situation appears to hold in both *Collinsia* and *Stylidium*. Hybridisation in the field is extremely rare, despite large numbers of populations that co-flower with congeners (Armbruster et al 2002; Juliet Wege, "personal communication"). Similarly, artificial hybrids can be made very rarely, suggesting there are strong postzygotic reproductive barriers (Armbruster "unpublished data"; Juliet Wege, "personal communication").

## Macroevolutionary shifts between pollination systems

Macroevolutionary data provide an additional means for evaluating our four hypotheses. Comparisons among closely related species can shed light on how transitions between pollinators occur over the course of evolutionary diversification and reveal relationships among species that differ in pollinators. In the context of reproductive isolation, it is important to understand how species-level shifts in pollination systems occur, and how often they might lead to reproductive isolation.

Contrary to predictions of the reproductive isolation hypotheses, speciation is not always tightly associated with shifts in pollinators. Examples can be found even in groups with specialized pollination. Well studied examples include species-rich groups such as *Pedicularis* (Orobanchaceae), which are almost all bumble-bee pollinated (Macior 1975, 1983; Yang et al. 2007), the Coryciine orchids that are pollinated by one genus of oil-collecting bees (Steiner 1989, Pauw 2006), and the genus *Burmeistera*, for which only one of more than one hundred speciation events is thought to be correlated with a shift in pollinators (Knox et al. 2008). These observations suggest that high speciation rates and increases in diversity often result from factors other than shifts between specialized pollinators (see also Hopper 1979; van der Niet et al 2006; Scopece et al. 2007; Cozzolino and Scopece 2008).

Even when evolutionary shifts between pollination systems do occur, we argue that they only rarely have the potential to cause reproductive isolation. Three kinds of pollinator shifts have been recognized (Armbruster 1993): (1) quantitative shifts, (2) qualitative shifts with an intermediate phase, and (3) qualitative shifts without an intermediate phase. Quantitative shifts occur through gradual changes in traits (often with quantitative-genetic inheritance) that influence the frequency of visitation by animal species and the probability that visitors pick up and deposit pollen. For example, an increase in the amount of nectar secreted might increase the rate of visitation by larger pollinators, but not a sudden transition from small to large (Heinrich and Raven 1972). An increase in the distance between the nectary and stigma lowers the probability that a small visitor contacts the stigma and deposits pollen compared to a larger visitor.

A study of *Dalechampia* vines (Euphorbiaceae) estimated 14 independent quantitative shifts in pollination systems (out of ca. 50 species studied). These involved shifts in blossom size and amounts of rewards offered, which influenced the rates of visitation and pollination by bee species of different body sizes. Because such shifts are probabilistic rather than complete, they are unlikely to generate sufficient reproductive isolation (in the absence of other barriers) to allow divergence of populations by drift or weak selection (Initial-RI) or to reinforce reproductive isolation (Reinforcement) in any but the weakest of manners.

Qualitative shifts with an intermediate phase (in which both old and new pollinators are effective; see Stebbins 1974) occur when the quality of the reward or advertisement has changed and causes a shift in pollinators. There is an intermediate phase in which both the old and new rewards and advertisements are present and both old and new pollinators visit. Thus, such shifts seem unlikely to generate sufficient reproductive isolation (in the absence of other barriers) to allow divergence of populations by drift or weak selection. Stebbins (1970, 1974) argued that this mode of pollinator shift, in combination with the quantitative shifts (which also involves an intermediate phase), was virtually the rule. Indeed some six out of seven qualitative pollinator shifts detected in *Dalechampia* involved an intermediate phase with both old and new rewards in place (Table 2).

Qualitative shifts without an intermediate phase involve a floral change that causes a complete switch to a new pollinator and immediate cessation of visitation by the old pollinator. This may occur frequently in orchids that are pollinated by male euglossine bees, and is thought to contribute to their high speciation rate and species diversity

**Table 2** Number of pollination shifts of each type observed in *Dalechampia*

| Type of pollination shift | Isolation consequences | Number observed |
|---|---|---|
| Quantitative shifts | No reproductive isolation | 15+ |
| Qualitative shifts with intermediate phase | No reproductive isolation | 5–6 |
| Qualitative shifts without intermediate phase | Immediate reproductive isolation, instantaneous speciation? | 1 |

(Dodson 1962; Dressler 1968, 1981; Chase and Hills 1992). However its frequency elsewhere in the angiosperms has not been considered. It is invoked in *Penstemon* (Straw 1955, 1956) and *Mimulus* (Schemske and Bradshaw 1999), but on fairly weak inference. It was found to be very rare in *Dalechampia* (1 out of ca. 22 documented shifts; Table 2; Armbruster 1993).

Less information is available in the other three study systems. Shifts between pollination systems across species appear to be largely quantitative in nature in *Collinsia* (interspecific variation in flower size), *Burmeistera* (interspecific variation in column length), and *Stylidium* (interspecific variation in column length and position; Armbruster et al. 1994; Wege 1999).

We suggest that these three types of pollinator shifts, as detected in comparisons of related species, are broadly applicable to all animal-pollinated plants. The relative frequency can inform our evaluation of the four possible causes of the diversity-specialization correlation. Only the third type of pollinator shift is likely to impart reproductive isolation and hence promote speciation. Its rarity in nature argues against the importance of Initial-RI and Reinforcement models.

## Phylogeographic patterns of sympatry

If either of the two reproductive-isolation hypotheses (Initial-RI or Reinforcement) is correct, we should expect sympatric species differing in pollination often to be sister species. This is because species are expected to diverge in pollination and achieve reproductive isolation in sympatry or parapatry. In contrast, there is no expectation of close relationship between sympatric species under the Extinction and Character-Displacement hypotheses. It is thus useful to examine the phylogenetic relationships of sympatric species.

Two or three *Dalechampia* species can often be observed growing together in the same habitat (see Armbruster 1985, 1986). Partitioning of pollinator resources is usually by attracting, and being pollinated by, different species of bees. Molecular phylogenetic and distributional data from *Dalechampia* indicate that sympatric species are almost never closely related. Most sister species usually occur in widely different places, with a mean separation of ca. 190 km, suggesting allopatric speciation without secondary sympatry of close relatives (Table 3). This argues against frequent sympatric or parapatric speciation, or reinforcement, by pollinator shifts in *Dalechampia*.

*Collinsia* assemblages often comprise two, or occasionally three, sympatric species. One instance of sibling species growing together and using different pollinators has been detected, but otherwise sympatric species are not usually closely related (Armbruster "unpublished data"). Sister species in the phylogeny tend to occur in geographically separate locations, with a mean separation of ca. 70 km (Table 3).

**Table 3** The number of pairs of sympatric species that are sister taxa and the mean geographical distance between allopatric sister species

| Taxon | No. species studied (no. sympatric pairs) | Number of sympatric sister species observed | Mean distance between allopatric sister species (±SE) |
|---|---|---|---|
| *Dalechampia* | 55 (ca. 95) | 2 | 187 (±24) km |
| *Collinsieae* | 24 (ca. 45) | 1 | 67 (±12) km |
| *Burmeistera* | 21 (6) | 0 | – |

*Burmeistera* assemblages typically comprise two to four co-flowering species (Muchhala and Potts 2007). Out of six sister species pairs in a phylogeny of 21 Ecuadorian species, none occurs sympatrically (Table 3; Knox et al. 2008). This phylogeny does not include species from other countries; however, should any of these turn out to be the actual sister species to the Ecuadorian pairs, it would not change the conclusion that these species do not occur sympatrically with their sister species.

Assemblages of *Stylidium* often comprise four or five co-flowering species. Sympatric species do not usually appear to be very closely related, judging from vegetative and reproductive differences (Armbruster et al. 1994, unpublished) and phylogenetic analyses (Wege 1999; Wege "personal communication"). Initial geographical and systematic evidence thus argues against frequent sympatric speciation by pollinator shifts (see also Hopper 1979; Coates et al. 2003).

Similar conclusions have been drawn from studies of members of the tribe Sinningieae (Gesnericaeae). Except in one small clade, sympatric species are rarely closely related, and phylogeographic evidence indicates that most speciation has occurred allopatrically (Perret et al. 2007). Thus for all of these study systems, phylogeographic data suggest that sister species are only rarely sympatric, supporting predictions of the Character Displacement and Extinction hypotheses over those of the two reproductive isolation hypotheses (see Table 1).

## Conclusions

Two tentative conclusions emerge from the data presented here: (1) There appears to be a relationship between clade species richness, floral specialization, floral integration, and adaptive accuracy. (2) Much of the covariance seems to have been generated by clade species richness influencing specialization rather than the reverse.

The comparative data from our four study systems provide varying support for the four models of the link between floral specialization and clade species richness. Most of the predictions of the Character-Displacement and Extinction models were borne out, whereas almost no predictions of the Initial-RI and Reinforcement models were supported (Table 1). Specifically, pollinators commonly move between species and pollination is only rarely extremely precise. With the possible exception of plants with pollinia (Orchidaceae and Asclepiadaceae), pollination is probably not sufficiently precise to completely eliminate interspecific pollen flow (see Muchhala and Potts 2007). In the study systems examined here, hybrids are rare, isolation appears to be largely post-zygotic, and sister species rarely occur sympatrically, suggesting speciation has been largely allopatric. Finally, pollinator shifts appear to have usually involved an intermediate phase, and thus could not have provided ethological isolation.

It seems likely that the association between clade species richness and specialized pollination is often the result of high species diversity selecting for the partitioning of pollinators through reproductive character displacement. The mechanism would be selection for high reproductive success in the face of reproductive interference (competition for pollination, sensu Waser 1983), even though post-zygotic reproductive isolation was already in place. Another important conclusion from our data is that the degree of floral integration and pollination accuracy affects the evolutionary response to this selective pressure. The relationship between floral traits and diversity may thus influence both steps of the evolutionary process: (1) the biotic environment (sympatric congeners and types of pollinators) selects for floral specialization, and (2) the degree of floral integration and precision determines the response to this selection.

The Character-Displacement hypothesis is also supported by the numerous reports of widespread plant species differentiating into pollination ecotypes (Dodson 1962; Grant and Grant 1965; Stebbins 1970; Miller 1981; Armbruster 1985; Inoue & Amano 1986; Pellmyr 1986; Steiner and Whitehead 1988, 1990, 1991; Robertson and Wyatt 1990; Susuki, 1992; Armbruster et al. 1994; Johnson 1994, 1997; Arroyo and Dafni 1995; Johnson and Steiner 1997; Muchhala and Potts 2007; see also Thompson 1994, 2005). This suggests that either differences in the local pollinator fauna, or more commonly "competition for pollination" (Waser 1983) select for floral differences (Dressler 1968; Whalen 1978; Howell 1977; Brown and Kodric-Brown 1979; Armbruster 1986, 1994; Nilsson et al. 1987, Goldblatt et al. 1995; Miyake and Inoue 2003; Tschapka et al. 2006; Muchhala and Potts 2007, Smith and Rausher 2007).

We are not arguing that the Initial-RI and Reinforcement models are invalid; indeed, these models may hold for some plant groups, like orchids with fused pollinia. Additional uncertainty relates to the importance of the Extinction model, although it has more stringent requirements than character displacement and hence may be rarer. The major conclusion here is that we need a pluralistic perspective to analyzing the correlation between floral specialization and species richness. We can view this as a problem in analysis of variance and covariance, in which we partition the covariance between specialization and diversity among at least four possible sources.

Our results reinforce Johnson's (2007) call that we increase our attention to the processes that drive divergence of populations and species as opposed to simply the factors contributing to reproductive isolation. This call is particularly relevant if it turns out that most features that appear to effect "reproductive isolation" are actually a product of selection for assortative pollination between populations that are already reproductively isolated (cf. Rieseberg and Willis 2007). Additional research is also needed to determine explicitly whether floral specialization increases or decreases the likelihood of extinction. Such an approach is especially timely, not only for evaluation of the hypotheses discussed here, but also because of the number of pollination mutualisms that are presently threatened by anthropogenic environmental change.

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