

Commentary

Quantum dots shed light on angiosperm speciation

For all sexual organisms, mate selection is an important factor in preventing gene flow between species. This is because it typically involves assortative mating: choosing mates similar to yourself and rejecting other possibilities. For animals, reproductive barriers during this stage are termed ‘behavioral isolation’, whereas for flowering plants the corresponding barriers are termed ‘floral isolation’. However, these are not simply interchangeable terms depending on your taxonomic preference, as the biological functions are fundamentally different. Most plants actually depend on another organism to find potential mates and ferry their pollen to them. Thus finding mates depends on intrinsic factors for animals, such as its own appearance and behavior, and extrinsic factors for flowering plants, such as the availability and behavior of pollinators. It is not immediately clear whether this distinction should amplify or decrease speciation rates for angiosperms. On the one hand, it should make angiosperms more susceptible to ecological speciation, or speciation due to divergent selection in different habitats: range expansions to include new habitats are not likely to affect animal mate-seeking behavior significantly, but these expansions can expose plants to very different pollinator regimes. On the other hand, the pollination process is inherently messy – to what extent can the behavior or morphology of pollinators actually restrict pollen movement between incipient species? Unfortunately, the earliest stages of speciation are notoriously difficult to study, and there is a dearth of evidence on the topic. In this issue of *New Phytologist*, Minnaar *et al.* (2019, pp. 1160–1170) provide one of the clearest demonstrations to date of floral isolation during incipient speciation, showing how evolutionary shifts in flower tube length serve to greatly reduce pollen transfer between long-

and short-tubed floral morphs. To track pollen movement, they debut a technique that Minnaar & Anderson (2019) recently developed to label pollen grains using quantum dots. The latest work by Minnaar *et al.* thus makes two contributions to the study of plant reproduction: it provides critical support for the potential role of floral isolation during speciation, and it illustrates the experimental use of an exciting new methodology, that has the potential to revolutionize the study of the male fitness component of plant reproductive success.

‘... how do we study the third step, the key moment when populations come back into secondary contact, which likely takes place in the geological blink-of-an-eye?’

Verne Grant (1949) was the first to clearly elucidate the potential role of pollinators during plant speciation (also see Kay & Sargent, 2009; Van der Niet *et al.*, 2014). Grant (1949) highlighted two main forms of floral isolation: ethological isolation, where pollinators specialize on only one floral morph and therefore do not move pollen between the morphs during foraging bouts; or mechanical isolation, where flower morphology prevents pollen transfer or results in pollen being placed on different parts of pollinators’ bodies (Fig. 1). Either or both of these can contribute to speciation according to the three-step scenario outlined in Fig. 2. In the first step, an angiosperm expands its geographic range into a region with unfamiliar local pollinator fauna. In the second step, the species evolves floral differences, either in parapatry or allopatry (i.e. along with a vicariance event that physically separates the populations). The third and final step is when the populations come into secondary contact and floral isolation serves to eliminate or

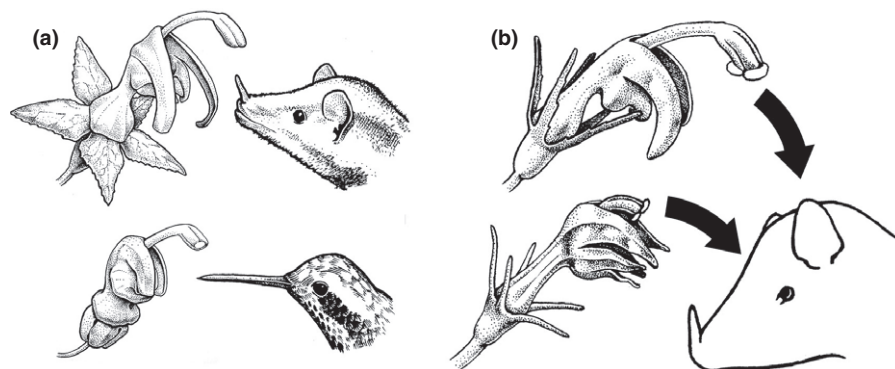


Fig. 1 The two subcomponents of floral isolation, illustrated with examples from the genus *Burmeistera* (Campanulaceae). (a) Ethological isolation between bat-adapted *B. borjensis* and hummingbird-adapted *B. rubrosepala* due to the attraction of different pollinators. (b) Mechanical isolation between *B. truncata* and *B. kitrinaima* due to differential pollen placement.

This article is a Commentary on Minnaar *et al.*, 2019, pp. 1160–1170.

greatly reduce pollen transfer between them, allowing the populations to continue on different evolutionary paths until they are fully distinct species. This intuitively satisfying scenario thus gives pollinators a starring role in the diversification of angiosperms.

Ever since Grant's (1949) seminal work, floral isolation has frequently been invoked to explain instances of shifts in diversification rates across angiosperm clades. Flowers that are more 'specialized;' in terms of, for example, radial versus bilateral symmetry, hummingbird versus generalist insect pollination, or the presence or absence of nectar spurs, are often associated with greater diversity than sister groups (Ree, 2005; O'Meara *et al.*, 2016). This squares nicely with the idea that such shifts amplify speciation by providing greater opportunity for pollinator-mediated reproductive isolation. However, of course, correlation does

not guarantee causation. For example, pollination by hummingbirds is much more prevalent at higher elevations in the Andes and in other Neotropical mountain ranges, where insect activity is less common. Thus the oft-cited pattern that hummingbird pollination correlates with increased diversity may, in fact, represent an incidental side effect of diversification driven by recent orogenesis and the many opportunities for allopatric speciation that mountains provide (Schmidt-Lebuhn *et al.*, 2019). In fact, the Andean uplift also greatly amplified lycophyte diversification rates (Testo *et al.*, 2019), which of course do not have flowers for pollinators to interact with (Lagomarsino, 2019). Furthermore, even distantly related taxa, long past the point of being able to exchange genes, will still undergo floral character displacement when they occur in sympatry because this reduces the costs of interspecific pollen transfer and competition for pollinators (Moreira-Hernández & Muchhala, 2019). Thus, it is possible that the causation may be reversed in the observed instances of associations between 'specialized' flowers and increased species diversity; in other words, members of particularly speciose clades frequently end up co-occurring, and selection then favors divergence in floral traits (Armbruster & Muchhala, 2009). Finally, the associations may actually represent specialized pollination causing a decrease in extinction rates, rather than an increase in speciation rates, because it allows populations to continue to reproduce at lower abundances.

To move beyond associations and gain a better understanding of the actual role of pollinators in angiosperm speciation, we need to focus our attention on the earliest stages of the process, when populations are beginning to diverge. Much evidence from a wide array of plants and pollination systems shows that flower morphology often evolves in a patchwork pattern of differences according to local pollinators, fulfilling the first and the second steps of Grant's (1949) floral isolation scenario (Van der Niet *et al.*, 2014). However, how do we study the third step, the key moment when populations come back into secondary contact, which likely takes place in the geological blink-of-an-eye? To be able to get a glimpse into a probable case of such secondary contact, Minnaar *et al.* took advantage of extensive prior work on the natural history of their focal plants, which had already shown the tight link between the tube lengths of *Laperiousia anceps* flowers and the proboscis lengths of their fly pollinators. Interestingly, their previous work also identified some populations with bimodal distributions of floral tube length, which is strongly suggestive of recent secondary contact between differently adapted populations. In the present study, Minnaar *et al.* pick one of these bimodal populations and examine whether floral isolation is effective enough to reduce gene flow between the morphs, and thus potentially contribute to speciation.

The results presented provide a positive answer to this question. Despite the same species of fly visiting flowers with two tube lengths in this population, and indiscriminately moving between them during foraging bouts, they transfer very little pollen between the morphs. Of the pollen that short-morph flowers received, 96% was from other short morphs (and only 4% from long morphs), while long-morph flowers received 78% from other long morphs (and only 22% from short morphs). Although we cannot know whether these populations will continue to remain distinct enough to speciate, this study provides support for the third step of Grant's

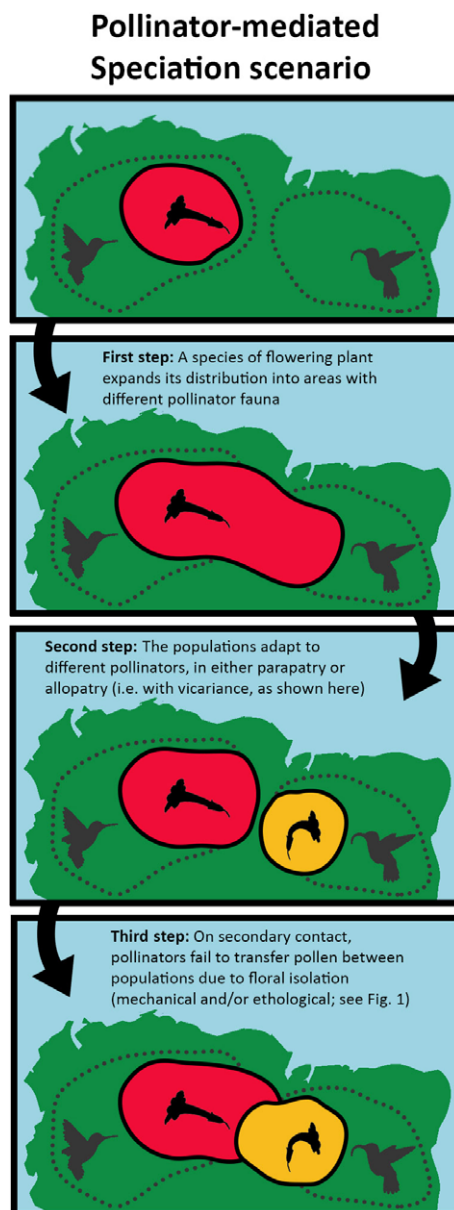


Fig. 2 Steps in Grant's (1949) scenario of pollinator-mediated speciation.

(1949) proposed pollinator-mediated speciation scenario, in that floral isolation (via mechanical isolation, rather than ethological isolation, in this case) can be strong enough on secondary contact to limit gene flow between populations.

To be able to track the movement of pollen grains, Minnaar *et al.* label pollen from the anthers of different flowers with quantum dots. Quantum dots are nanoparticles that glow in different colors under a fluorescent microscope (Minnaar & Anderson, 2019). This method solves a critical problem in pollination biology – the female component of fitness is easy to quantify by counting seed production or pollen that arrives to stigmas, however, how can we measure male fitness in terms of how many pollen grains an individual successfully disperses to receptive females? Pollen transfer rates can be estimated by tracking the movement of fluorescent dye powder (Waser & Price, 1982), but tracking the movement of pollen grains themselves is notoriously tricky. The only studies that successfully achieve this genotyped individual pollen grains with microsatellites (Hasegawa *et al.*, 2009), or relied on the rare instances of intraspecific polymorphisms in pollen color (Thomson & Plowright, 1980), or intrageneric differences in pollen morphology that allows for species identification (Muchhala & Potts, 2007). In fact, this difficulty in tracking pollen may go a long way towards explaining the lack of studies that explore ethological and (especially) mechanical isolation. Thus Minnaar *et al.* provide an important tool for studying the role of pollinators in angiosperm speciation, one that I anticipate will greatly impact the whole field of plant reproductive biology, allowing us to study male fitness with much greater precision than past techniques afforded.

One detail in Minnaar *et al.*'s results that may be initially surprising is the asymmetry in pollen transfer between the floral morphs, in that one might expect pollen to move at equal rates in both directions. However, careful observation of the mechanics of flower–fly fit elucidates this pattern. A pollinating fly extends its proboscis fully during visits to the flower, and exerts force until the proboscis reaches the nectar at the base of the flowers, thus short-morph pollen ends up placed closer to the tip of the proboscis, while long-morph pollen ends up further back on the proboscis and on the fly's head. When the fly visits the next flower, short-morph pollen occasionally ends up on long-morph stigmas as the proboscis scrapes by, but the reverse occurs very rarely. In fact, the (admittedly small) handful of studies that have carefully tracked pollen transfer between species consistently documents such asymmetry (e.g. Wolf *et al.*, 2001). When we consider this on a broader scale, the strength of other reproductive barriers to gene flow between co-occurring species (i.e. low germination of foreign pollen grains, stunted growth of foreign pollen tubes, decreased hybrid zygote formation, and low hybrid viability) similarly tends to vary sharply depending on which species is the pollen donor and which is the pollen recipient (Moreira-Hernández & Muchhala, 2019). This demonstrates that such asymmetry in barrier strength is the norm, rather than the exception.

In summary, Minnaar *et al.* provide a rare glimpse into the potential role of floral isolation during the earliest stages of angiosperm diversification. Although such studies have increased in number in recent years, still more are needed to fully understand the extent to which pollinators drive ecological speciation in plants.

Possible approaches include finding putative sites of recent secondary contact, such as Minnaar *et al.* have done here (also see, for example, Natalis & Wesselingh, 2013), experimentally bringing different populations into 'secondary contact' in common gardens (e.g. Kay *et al.*, 2018), or employing extensive analyses across a clade in combination with a time-calibrated phylogeny to analyze the order of appearance of different isolating factors (e.g. Kostyun & Moyle, 2017). I expect that Minnaar *et al.*'s elegant work will inspire further studies of floral isolation during speciation, and will serve to demonstrate an exciting new tool that holds promise for studies of all aspects of the male fitness component of plant reproduction.

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References

- Armbruster WS, Muchhala N. 2009. Associations between floral specialization and species diversity: cause, effect, or correlation? *Evolutionary Ecology* 23: 159–179.
- Grant V. 1949. Pollination systems as isolating mechanisms in angiosperms. *Evolution* 3: 82–97.
- Hasegawa Y, Suyama Y, Seiwa K. 2009. Pollen donor composition during the early phases of reproduction revealed by DNA genotyping of pollen grains and seeds of *Castanea crenata*. *New Phytologist* 182: 994–1002.
- Kay KM, Sargent RD. 2009. The role of animal pollinators in plant speciation: Integrating ecology, geography, and genetics. *Annual Review of Ecology, Evolution, and Systematics* 40: 637–656.
- Kay KM, Zepeda AM, Raguso RA. 2018. Experimental sympatry reveals geographic variation in floral isolation by hawkmoths. *Annals of Botany* 123: 405–413.
- Kostyun JL, Moyle LC. 2017. Multiple strong postmating and intrinsic postzygotic reproductive barriers isolate florally diverse species of *Jaltomata* (Solanaceae). *Evolution* 71: 1556–1571.
- Lagomarsino LP. 2019. Rapid plant diversification in the Andes does not require flowers. *New Phytologist* 222: 11–13.
- Minnaar C, Anderson B. 2019. Using quantum dots as pollen labels to track the fates of individual pollen grains. *Methods in Ecology and Evolution* 10: 604–614.
- Minnaar C, de Jager ML, Anderson B. 2019. Intraspecific divergence in floral-tube length promotes asymmetric pollen movement and reproductive isolation. *New Phytologist* 224: 1160–1170.
- Moreira-Hernández JI, Muchhala N. 2019. Importance of pollinator-mediated interspecific pollen transfer for angiosperm evolution. *Annual Review of Ecology, Evolution, and Systematics* 50: 8.1–8.27.
- Muchhala N, Potts MD. 2007. Character displacement among bat-pollinated flowers of the genus *Burmeistera*: analysis of mechanism, process and pattern. *Proceedings of the Royal Society B* 274: 2731–2737.
- Natalis LC, Wesselingh RA. 2013. Parental frequencies and spatial configuration shape bumblebee behavior and floral isolation in hybridizing *Rhinanthus*. *Evolution* 67: 1692–1705.

- O'Meara BC, Smith SD, Armbruster WS, Harder LD, Hardy CR, Hileman LC, Hufford L, Litt A, Magallón S, Smith SA. 2016. Non-equilibrium dynamics and floral trait interactions shape extant angiosperm diversity. *Proceedings of the Royal Society B: Biological Sciences* **283**: 20152304.
- Ree RH. 2005. Detecting the historical signature of key innovations using stochastic models of character evolution and cladogenesis. *Evolution* **59**: 257–265.
- Schmidt-Lebuhn AN, Müller M, Pozo Inofuentes P, Encinas Viso F, Kessler M. 2019. Pollen analogues are transported across greater distances in bee-pollinated than in hummingbird-pollinated species of *Justicia* (Acanthaceae). *Biotropica* **51**: 99–103.
- Testo WL, Sessa E, Barrington DS. 2019. The rise of the Andes promoted rapid diversification in Neotropical *Phlegmariurus* (Lycopodiaceae). *New Phytologist* **222**: 604–613.
- Thomson JD, Plowright R. 1980. Pollen carryover, nectar rewards, and pollinator behavior with special reference to *Diervilla lonicera*. *Oecologia* **46**: 68–74.
- Van der Niet T, Peakall R, Johnson SD. 2014. Pollinator-driven ecological speciation in plants: new evidence and future perspectives. *Annals of Botany* **113**: 199–212.
- Waser NM, Price MV. 1982. A comparison of pollen and fluorescent dye carry-over by natural pollinators of *Ipomopsis aggregata* (Polemoniaceae). *Ecology* **63**: 1168–1172.
- Wolf PG, Campbell DR, Waser NM, Sipes SD, Toler TR, Archibald JK. 2001. Tests of pre- and postpollination barriers to hybridization between sympatric species of *Ipomopsis* (Polemoniaceae). *American Journal of Botany* **88**: 213–219.
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