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## Pollen transport: Illuminating a key mechanism of disassortative pollination

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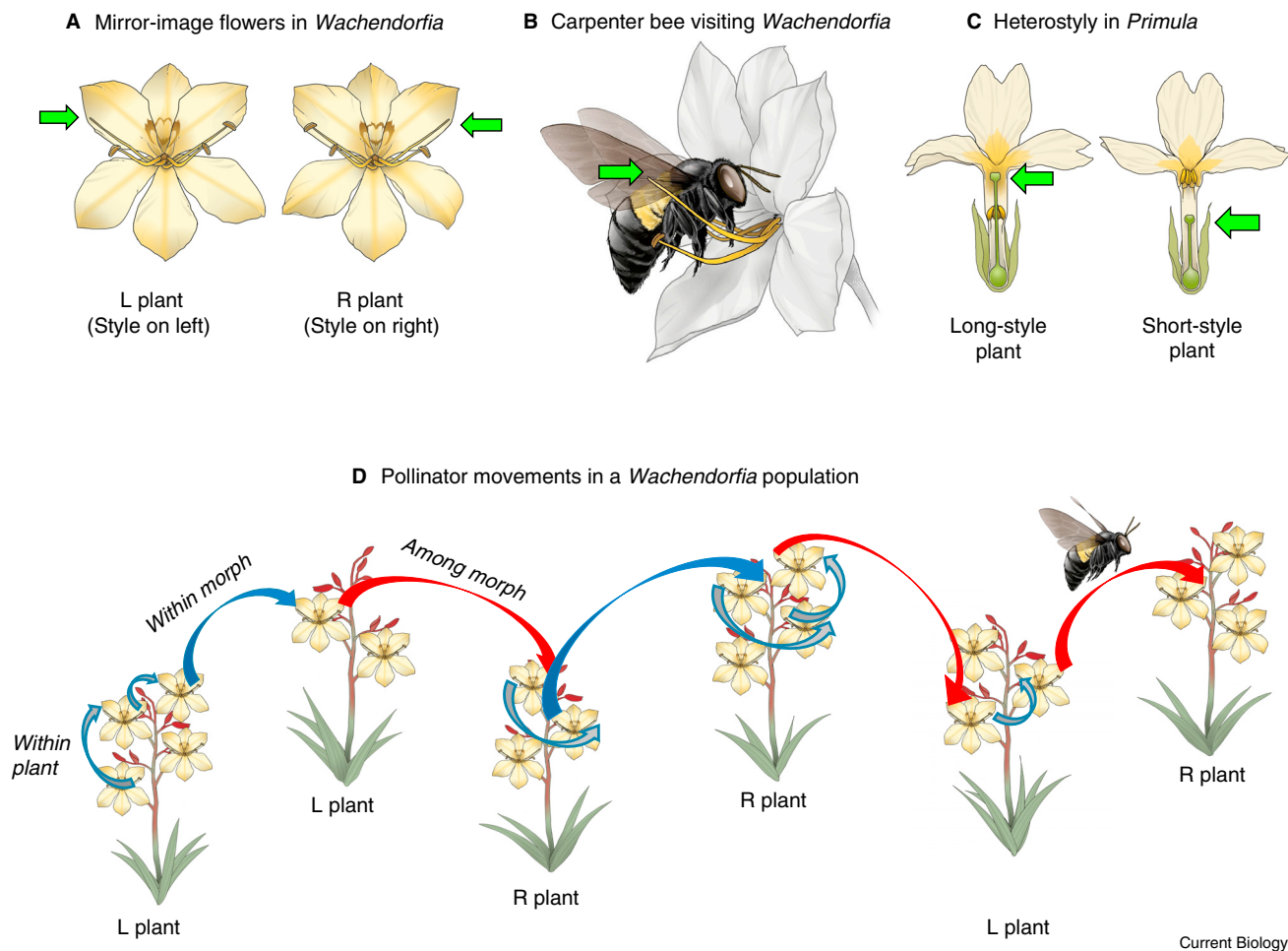
Floral sexual polymorphisms have evolved repeatedly in angiosperms and are thought to reduce self-pollination and increase pollen export. Using a powerful pollen-labeling technique, quantum dots, a new study shows that pollen placement on pollinator bodies plays a critical role in disassortative pollination.

Most flowering plants rely on animal vectors to transfer pollen to potential mates<sup>1</sup>. However, patterns of pollinator foraging often fail to optimize mating from an individual plant's perspective. Pollinators typically visit multiple flowers on each plant and therefore may transfer pollen to adjacent flowers on a floral display<sup>2</sup>. Since flowers of most angiosperm species are 'cosexual', bearing both male and female organs, pollinator movements between flowers on the same plant increase the likelihood of self-fertilization<sup>3</sup>. This extreme form of inbreeding reduces female fitness due to inbreeding depression. Transfer of self-pollen to

adjacent flowers also wastes pollen that could have been exported to other plants, and consequently reduces male fitness<sup>4</sup>. Although among-flower, within-plant self-fertilization is often considered to be an unavoidable consequence of an increase in plant size<sup>5</sup>, some flowering plants have evolved floral morphologies that lessen the likelihood of this form of self-pollination. Perhaps the most intriguing strategies are polymorphisms in the arrangement of sex organs within a flower, and these have arisen repeatedly in the angiosperms<sup>6</sup>. Floral sexual polymorphisms fascinated Charles Darwin<sup>7</sup>, who referred to them as a

"most complex marriage arrangement". By promoting disassortative pollination between the two floral forms, or morphs, these sexual polymorphisms reduce self-fertilization within and among flowers on individual plants and promote pollen export to other individuals in the population. Extensive research has explored the genetic basis of sexual polymorphisms, the evolution and breakdown of these polymorphisms, and the extent of pollen transfer within and amongst morphs<sup>8</sup>. However, the intricate pollination mechanisms leading to the resulting mating patterns remain largely unexplored<sup>9</sup>. In a new study published in this issue of *Current*





**Figure 1. Floral sexual polymorphisms reduce self-pollination and increase pollen export because reproductive structures among morphs contact different locations on pollinator bodies.**

(A) Mirror-image flowers of *Wachendorfia paniculata* differ in the position of the style and upper anther (enantiostyly). (B) A carpenter bee with pollen on its right wing contacts the stigma of a right-styled morph of *W. paniculata*. This promotes disassortative pollination between morphs. (C) Heterostyly, studied extensively in *Primula*, is a sexual polymorphism characterized by reciprocal placement of the style and anthers. Quantum dots can be used to examine patterns of pollen placement in heterostylous species. (D) When a pollinator forages in a population of enantiostylous plants, sequential intramorph visits may deposit only small quantities of pollen, resulting in increased pollen dispersal distances. Quantum dots can be used to document the shape of the pollen carryover curve. (Illustrations: Alysa Hallett and Jeffrey Karron.)

*Biology*, Corneile Minnaar and Bruce Anderson<sup>10</sup> use an innovative pollen labeling and mapping technique to document how the spatial location of pollen deposited by each anther on the bodies of wild bees influences the extent of self-fertilization and disassortative mating.

This elegant research explored the mechanics of the pollination process in *Wachendorfia paniculata*, a self-compatible bee-pollinated plant native to South Africa. *Wachendorfia* populations have an unusual sexual polymorphism known as ‘mirror-image flowers’ or dimorphic enantiostyly<sup>11</sup>. Roughly half

the plants in each population have the female reproductive structure (style) deflected to the left, and the other half have the style deflected to the right<sup>12</sup> (Figure 1A). All flowers on each plant are either ‘left-styled’ or ‘right-styled’, and research on another species with mirror-image flowers suggests that this sexual polymorphism is under simple genetic control<sup>13</sup>. Previous researchers studying *Wachendorfia* hypothesized that differential pollen placement on the left or right side of bee pollinators lessens the likelihood of among-flower self-fertilization and promotes cross-pollination<sup>11,12</sup>.

To map how the left-styled and right-styled floral morphs deposit pollen on bees, Minnaar and Anderson<sup>10</sup> labeled pollen grains on the three anthers of each morph with different colors of quantum dots, fluorescent semiconductor nanocrystals that emit colors under UV excitation<sup>14</sup>. The quantum dots adhere directly to pollen grains and therefore do not influence pollen placement or pollen transport<sup>15</sup>. *Wachendorfia* plants were then visited by two bee species that differ in body size and therefore in positions of pollen placement. When *Wachendorfia* flowers were visited by large-bodied carpenter bees (*Xylocopa caffra*), the

lower anthers primarily deposited pollen on the abdomen, whereas the upper anthers deposited pollen on the middle region of the wings. Since the female reproductive structure of the mirror-image morphs is positioned to collect pollen primarily from the wings (Figure 1B), carpenter bees are highly effective at facilitating disassortative pollination. By contrast, honey bees (*Apis mellifera capensis*) have a smaller body size and received little pollen from the upper anthers. Therefore, honey bees are much less effective at promoting disassortative pollination. This suggests that differences in pollinator composition could lead to differences in selfing rate<sup>16</sup>.

In a second experiment, Minnaar and Anderson<sup>10</sup> provide compelling evidence that relatively little self-pollen is transferred among *Wachendorfia* flowers on an individual plant. This was accomplished by labeling all of the anthers on a plant with a single quantum dot color and then labelling other plants with different colors. Only 8% of pollen transfer occurred among flowers on an individual plant. Therefore, this sexual polymorphism is very effective at promoting cross-fertilization.

Distinct regions of *Wachendorfia* pollen placement by the two morphs are analogous to the differential placement of pollen from distylous *Primula vulgaris* (Figure 1C) on distal and proximal regions of the body of bee pollinators<sup>17</sup>. Therefore, quantum dot studies would be extremely valuable for heterostylous species, which have a polymorphism characterized by reciprocal placement of the style and anthers, as these studies could elucidate the effectiveness of heterostyly in limiting the amount of within-plant self-pollination. Most heterostylous species have heteromorphic incompatibility, which prevents self-fertilization following within-plant self-pollination<sup>8</sup>. However, some heterostylous species, such as *Primula oreodoxa*, lack heteromorphic incompatibility<sup>18</sup>. This sub-alpine species from Sichuan, China has populations with both homostylous and heterostylous morphs. It would be interesting to use quantum dots to explore how among-flower, within-plant self-pollination varies between these morphs.

How else can we utilize Minnaar and Anderson's novel pollen-labeling

technology? Sexual polymorphisms also have important implications for patterns of gene dispersal and the genetic structure of populations. When a pollinator visits a focal plant, pollen from that plant is typically deposited in declining quantities onto stigmas of the next several plants in the visitation sequence<sup>19</sup>. However, since enantiostyly promotes among-morph pollen transfer, sequential intramorph visits may deposit small quantities of pollen, resulting in increased pollen carryover (Figure 1D). Longer pollen dispersal distances and larger genetic neighborhoods lessen the likelihood of biparental inbreeding (for example, matings between siblings or cousins). This could readily be tested experimentally using quantum dots to document the carryover curve with varying frequencies of different mirror-image morphs.

Flowering plants have evolved many reproductive strategies to influence pollinator behavior and promote cross-fertilization. Minnaar and Anderson<sup>10</sup> provide one of the first studies to elucidate the mechanism by which a sexual polymorphism leads to disassortative pollination, resulting in decreased rates of self-fertilization. By labeling individual pollen grains, they showed how anthers of right-styled flowers precisely deposit pollen in a location on the pollinator's body that corresponds to where the stigma of the left-styled morph contacts the pollinator, and vice versa. The quantum dot technology has opened exciting new avenues of research into fine-scale studies of pollinator-mediated pollen transfer.

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