

## Commentary

### Catching on to concatenation: evidence for pre-pollination intra-sexual selection in plants

Darwin (1871) proposed the mechanism of sexual selection to explain the extreme traits, secondary sex characters, seen primarily in male animals for either display or competition. These elaborate traits for attracting members of the opposite sex or competing for access to mates, inter- and intra-sexual selection, respectively, would increase the mating success of individuals but may reduce individual survival. Sexual selection has traditionally been associated with motile, dioecious animals that have well-developed sensory abilities and has long been viewed as absent as a mode of selection in plants (Grant, 1995). However, since the 1980s, several workers have argued that sexual selection is applicable to plants and should be considered as a factor in floral evolution (Skogsmyr & Lankinen, 2002). Opponents such as Grant (1995) point to incompatibility with Darwin's usage, hermaphroditism, the absence of obvious secondary sex characters and the apparent absence of female choice in plants (Skogsmyr & Lankinen, 2002; Moore & Pannell, 2011). Proponents, however, argue that historical definitions should be expanded to explain modern evidence and that sexual selection can, in principle, occur in hermaphrodites and hence play a role in the evolution of floral traits. The latter view is supported by a recent study by Cocucci *et al.* (this issue of *New Phytologist*, pp. 280–286) which demonstrates intra-sexual selection in plants via direct male–male competition between the pollinaria of milkweeds for optimal attachment sites on pollinators to ensure subsequent pollinium deposition. This is the first example of male–male competition resulting in secondary sex characters in hermaphrodite plants, highlighting the possibility of overlooked mechanisms of sexual selection in plants.

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In milkweeds (Asclepiadoideae; Apocynaceae), the androecium and gynoecium are fused, forming a single actinomorphic structure, the gynostegium, bearing parallel pairs of guide rails

which form five stigmatic slits situated below five pollinaria. Pollinaria consist of two pollinia from adjacent anthers, attached via caudicles to a clip-like structure, the corpusculum, positioned at the top of each stigmatic slit. When a limb, proboscis or other pollinator appendage is drawn between the guide rails of the stigmatic slit, a pollinarium may be mechanically clipped onto the pollinator. Pollen deposition occurs in a similar way, except that a pollinium or part thereof, rather than a pollinator appendage, may be drawn between the guide rails and broken off in the stigmatic chamber. In most members of the tribe Asclepiadeae, the entire pollinium is deposited, whereas in the Ceropegieae (including stapeliads) and Marsdenieae, only part of the pollinium, the insertion crest, is inserted. In some species, the caudicle may collect another pollinarium after the deposition of a pollinium, a process termed concatenation, resulting in the formation of elaborate chains or agglomerations of pollinaria on pollinators (Morse, 1981; Coombs *et al.*, 2009, 2012; Wiemer *et al.*, 2012; Cocucci *et al.*).

The evolutionary importance of pollinaria chains has seldom been considered, although long chains may decrease foraging efficiency of bumblebees (Morse, 1981). Recently, Coombs *et al.* (2012) found no effect of large agglomerations of *Cynanchum ellipticum* pollinaria on the foraging efficiency of visiting bees, but suggested that concatenation could benefit the plant by increasing the number of attachment sites on a pollinator. In this issue, Cocucci *et al.* show that for New World Oxypetalinae, the terminal pollinaria in the chains formed on pollinators are in a favourable position to deposit pollinia on stigmas while proximal pollinia are at a disadvantage. They then provide empirical and phylogenetic evidence to support the hypothesis that in some species, subtle adaptations of the caudicle to bear horn-like structures are examples of secondary sex characters. These caudicle horns apparently function to prevent the formation of chains and are likely to have arisen through pre-pollination, male–male competition.

The formation of pollinaria chains appears to be particularly prevalent in New World asclepiads (Morse, 1981; Wiemer *et al.*, 2012; Cocucci *et al.*). The phenomenon has been reported from only two African asclepiads, *Cynanchum ellipticum* and *Gomphocarpus physocarpus*, and appears to be associated with a relatively imprecise, haphazard mechanism of pollinium insertion and species with generalist pollination systems (Coombs *et al.*, 2009, 2012). Concatenation has not been observed in other African Asclepiadoideae with which we are familiar (e.g. *Asclepias*, *Pachycarpus*, *Xysmalobium* and various stapeliads). The absence of concatenation in these African groups does not appear to be associated with similar caudicle horns as described by Cocucci *et al.* and a survey of 97 species of Asclepiadeae across seven genera (illustrated in various publications) revealed none that had pollinarium adaptations comparable to the South American taxa (Fig. 1c). One African group in which ornate caudicles are widespread, is the stapeliads (Ceropegieae; Bruyns, 2005; Fig. 1d),

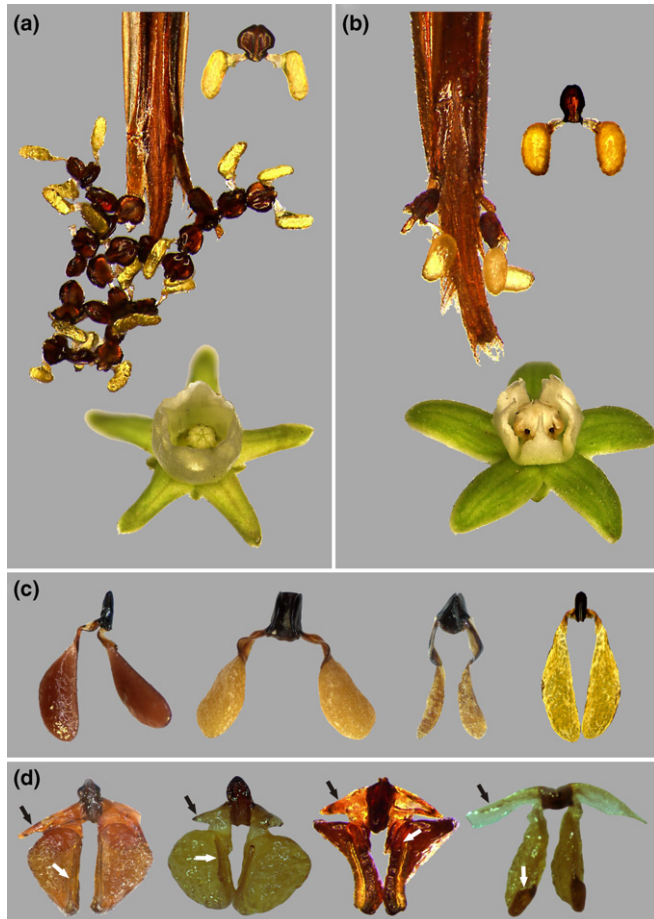
but in these flowers only the insertion crest of the pollinium is inserted, preventing the removal of pollinaria by caudicles and hence precluding concatenation. In stapeliads, ornate caudicles possibly function to enforce precise orientation of the pollinium for insertion. Although the mechanism preventing concatenation in many of these systems is not clear, the widespread absence of concatenation, combined with the results of Cocucci *et al.*'s study,

suggests that there are costs associated with concatenation, most likely through differences in siring success of proximal vs distal pollinaria in chains.

Cocucci *et al.* point out that selection could favour concatenation if most of the pollinaria in a chain originate from one plant, as concatenation could increase the number of attachment sites on pollinators. This is likely the case in the South African *Cynanchum ellipticum*, which produces numerous flowers (Coombs *et al.*, 2012). However, collecting multiple pollinaria from one plant would also be expected to increase rates of geitonogamous self-insertions, adding further complexity to the interaction. Interestingly, a congener of *C. ellipticum*, *C. obtusifolium*, also pollinated by native honey bees and with similar floral morphology to *C. ellipticum*, rarely forms pollinaria chains on pollinators (compare Fig. 1a and b). Again, this implies the existence of trade-offs controlling the evolution of fine-scale mechanisms of pollinium transfer between flowers.

The apparent absence of morphological adaptations preventing concatenation in many asclepiads suggests that such adaptations may be subtle (e.g. pollinium orientation) and consequently overlooked. Another possibility is that the orientation of insects for pollinium insertion may be more precise in some systems than in others. There is some evidence that New World asclepiads have considerably more generalized pollination systems than African asclepiads (Kephart, 1983; Fishbein & Venable, 1996). Indeed, even the wasp-pollinated taxa studied by Wiemer *et al.* (2012) are more generalized than comparable South African wasp-pollinated asclepiads (Shuttleworth & Johnson, 2009, 2012). Flowers of asclepiads with highly specialized pollination systems exhibit elaborate coronal morphology with extended lobes and various horns (cf. members of the genera *Pachycarpus* and *Miraglossum* illustrated in Shuttleworth & Johnson, 2012). These elaborate morphological structures appear to function as adaptations to position pollinators precisely for the placement of pollinaria on specific body parts (Ollerton *et al.*, 2003). Similar coronal adaptations are seldom observed in species with more generalized pollination systems, including New World *Asclepias* and African *Gomphocarpus physocarpus*. In these systems, pollinium removal and deposition is often haphazard and involves various body parts (Morse, 1981; Kephart, 1983; Coombs *et al.*, 2009). Is it possible that the evolution of morphological adaptations associated with highly specialized pollination systems in African asclepiads is driven by male–male competition associated with the costs of concatenation? At this stage it is difficult to assess, but the results of Cocucci *et al.*'s study suggest that intra-sexual competition between the male function of plants may have played an important role in the fine scale evolution of pollination mechanisms in milkweeds.

Sexual conflict may occur in other hermaphroditic plants, particularly those, such as orchids, with pollen aggregated as pollinia. Orchid flowers are typically highly specialized for pollination by few species of pollinators and often show adaptations for precisely orienting pollinators and loading pollinaria onto specific body parts. In a few species, very large numbers of pollinaria may be precisely deposited onto specific parts of pollinators including compound eyes (Johnson & Liltved, 1997), frons (Peter



**Fig. 1** Comparison of pollinarium attachment to pollinators between two species of *Cynanchum* and diversity of pollinarium morphologies in African Asclepiadoideae. (a) *Cynanchum ellipticum* pollinaria attach to proboscides of pollinating honey bees and frequently concatenate into extensive chains which ultimately form tangled agglomerations of pollinaria. (b) The pollinaria of *Cynanchum obtusifolium*, by contrast, rarely form chains and individual pollinaria are attached directly to the mouth parts. Note the difference in coronal morphology between *C. ellipticum* and *C. obtusifolium*: in the former, the corona forms an undifferentiated cup surrounding the gynostegium. Pollinators probe haphazardly to access nectar at the base of the cup and randomly collect pollinaria on their mouthparts. In the latter, the corona has small horns which may guide the probing pollinators and possibly confer a more precise placement of pollinaria on the proboscis. (c) Examples of pollinaria lacking caudicle horns from the African Asclepiadoideae include (from left) *Asclepias albens*, *Pachycarpus concolor*, *P. appendiculatus* and *Gomphocarpus physocarpus*. (d) By contrast, many species of stapeliads (Ceropegieae) do have horn-like structures on their caudicles (black arrows) but these are thought to serve functions besides preventing concatenation as in these species only a portion of the pollinium, the insertion crest (white arrows), enters the stigmatic slit. Examples include (from left) *Stapelia gigantea*, *Orbea hardyi*, *O. verrucosa* and *Duvalia polita*. (Images not to scale.)

& Johnson, 2009) and the base of proboscides (Johnson *et al.*, 2005). In this latter case, pollinaria are attached sequentially to the proboscides of pollinating hawkmoths and the distal position of overlying pollinaria is thought to be favourable for subsequent deposition on stigmas (Johnson *et al.*, 2005). This suggests that in some orchids, the presence of a large number of pollinaria and a favourable position for pollinaria on pollinators are comparable to the hypothesized conditions for male–male competition proposed by Cocucci *et al.* In addition, new experimental evidence suggests that male interference can reduce pollination efficiency and seed set in an orchid specialized for hawkmoth pollination (Duffy & Johnson, 2014). The results of Cocucci *et al.* are consistent with the interpretation of Romero & Nelson (1986) that the explosive emplacement of pollinaria on pollinating Euglossine bees by male flowers of various dioecious species of *Catasetum* and *Cycnoches* serves to negatively condition the bees that hence avoid other male flowers, but not female flowers. While the mechanism is different, the outcome is the same with pollinators rendered unusable by other male flowers, but not female flowers.

Direct pre-pollination male–male competition in other angiosperms seems less likely. However, it is plausible that such competition might occur in species with specialized pollination systems where pollen is accurately loaded onto specific parts of pollinators. The challenge remains to identify situations in which pollen from different individuals may experience differential success and then identify cryptic traits that could function to ameliorate differences in siring success between individuals.

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