

Opinion piece

On the problems of a closed marriage: celebrating Darwin 200

Darwin devoted much of his working life to the study of plant reproductive systems. He recognized that many of the intricacies of floral morphology had been shaped by natural selection in favour of outcrossing, and he clearly established the deleterious effects of self-fertilization on progeny. Although Darwin hypothesized the adaptive significance of self-fertilization under conditions of low mate availability, he held that a strategy of pure selfing would be strongly disadvantageous in the long term. Here, I briefly review these contributions to our understanding of plant reproduction. I then suggest that investigating two very different sexual systems, one in plants and the other in animals, would throw further light on the long-term implications of a commitment to reproduction exclusively by selfing.

Keywords: sexual systems; cleistogamy; androdioecy

1. INTRODUCTION

Although he modestly denied it, Darwin was as much a botanist as a zoologist, publishing no fewer than six books on plants. Three of these focused on aspects of plant reproductive systems: the first book on the floral adaptations of orchids for outcrossing (Darwin 1862); the second on the effects of self-fertilization versus outcrossing (Darwin 1876); and the third on plants with polymorphic or heteromorphic floral characters (Darwin 1877). Darwin came to view plants as a wonderful illustration of the action of natural selection in shaping morphological characters that had hitherto been regarded as inconsequential to a plant's needs. He derived enormous satisfaction in inferring their functional significance, and he once claimed that nothing in his scientific life had given him 'so much satisfaction as making out the meaning of the structure of heterostyled flowers', to which he devoted the greater part of his book on *The different forms of flowers on plants of the same species* (Darwin 1877; in heterostylous populations, half the individuals have long styles and short stamens while the other half have short styles and long stamens).

Two related themes run through Darwin's work on plant sexuality: the mechanisms that plants have evolved to promote outcrossing and the relative disadvantages and advantages of self-fertilization. In this paper, I comment on Darwin's work on inbreeding depression and his insight into plants that reproduce exclusively by selfing in the long term would run into problems. Consistent with this idea,

most plants that have evolved a syndrome of self-fertilization nevertheless do cross with one another from time to time. The maintenance of an ability to cross in otherwise highly selfing populations also applies to plants that have evolved completely closed flowers, as well as to hermaphroditic animals that physically cannot mate with one another. Further research on these two reproductive systems might help us to understand the extent to which the short-term benefits of self-fertilization trade-off against its long-term disadvantages.

2. THE ABHORRENCE OF PERPETUAL SELFING

In the years following the publication of *On the origin of species* (Darwin 1859), Darwin turned to the completion of his book *On the various contrivances by which British and foreign orchids are fertilised by insects* (Darwin 1862), in which he exposed the functional significance of many of the intricacies of orchid flowers. Although the German botanist Konrad Sprengel had published an account of the function of flowers nearly 70 years earlier (Sprengel 1793), it was Darwin who first fully recognized that hermaphrodite flowers had been shaped by natural selection largely to enhance cross-fertilization and to avoid selfing. His observations led him to conclude in *Orchids* the inference that nature 'abhors perpetual self-fertilization' (p. 359).

Fourteen years later, Darwin published the results of experiments conducted in his 'hothouse' at Down: *The effects of cross and self-fertilisation in the vegetable kingdom* (Darwin 1876). These experiments were largely motivated by a search for why natural selection had repeatedly favoured characters in plants that ensured crossing and prevented inbreeding, but Darwin was possibly also influenced by his anxiety that his marriage to his cousin, Emma Wedgwood, might have been responsible for the poor health of his children (Jones 2008). Ultimately, however, it was serendipity that got him started: he noted after selfing and crossing individuals of the toadflax *Linaria vulgaris*, '[f]or the sake of determining certain points with respect to inheritance' (Darwin 1876, p. 9), that the selfed progeny performed less well than the crossed.

Darwin repeated the comparison of inbred and outbred individuals for numerous other plant species by pitting selfed versus outcrossed progeny against one another in carefully controlled experiments. He documented inbreeding depression under a range of experimental conditions and recognized that its expression depends very much on the environment under which progeny are grown. The environmental dependency of inbreeding depression is now well established (e.g. Dudash 1990) and may contribute to the maintenance of intermediate outcrossing rates in plants (reviewed in Goodwillie *et al.* 2005). In the course of his experiments, Darwin (1876) also stumbled upon what we now recognize as self-incompatibility. Intriguingly, although he recognized the adaptive significance of morphological traits that prevent selfing, he viewed self-infertility as somehow an 'incidental' trait with no obvious adaptive significance (p. 346).

Without our modern understanding of genetics, it is hard to imagine how one might explain inbreeding

depression; Darwin's (1876) explanation, which invoked an intrinsic advantage of crossing between individuals with somewhat divergent phenotypes, was thus understandably vague. Nor could Darwin explain his observation of what we would call the purging of inbreeding depression. Nonetheless, by subjecting his material to multiple generations of artificial selfing, he almost certainly discovered the phenomenon long before it was clearly confirmed in more recent times (Barrett & Charlesworth 1991). After six generations of selfing and crossing lineages of the plant *Ipomoea purpurea*, he noted that one selfed lineage became stronger than all the others. Amusingly, he referred to this lineage as 'the Hero', 'which after a long and dubious struggle conquered its crossed opponent, though by only half an inch!' (Darwin 1876, p. 47).

Darwin's observations convinced him that self-fertilization was intrinsically deleterious. However, he also recognized that the ability to self in the absence of mates could be an advantage, noting that hermaphrodites might benefit from 'occasional or frequent self-fertilization, so as to ensure the propagation of the species, more especially in the case of organisms affixed for life to the same spot' (Darwin 1876, p. 462). The selection of self-fertile hermaphroditism for reproductive assurance is now widely accepted as responsible for the breakdown of self-incompatibility or the evolution of hermaphroditism from dioecy in many plant and animal species (Eppley & Jesson 2008), particularly those with a history of long-distance dispersal (Baker 1955).

Yet Darwin could not believe that complete selfing in the absence of any outcrossing could be maintained in the long term. His intuition is borne out by the fact that even habitual selfers, such as the model species *Arabidopsis thaliana*, do occasionally outcross and show evidence in their genomes of recombination and its benefits (reviewed in Wright *et al.* 2008). For instance, a small amount of outcrossing in habitual selfers probably accounts for the relatively rapid decline in linkage disequilibrium across the genome and is sufficient to overcome the negative implications of what would otherwise be an absence of effective recombination, such as the accumulation of deleterious mutations and a slowdown in the rate of adaptation (reviewed in Wright *et al.* 2008).

3. CLEISTOGAMOUS PLANTS: NOT A TRULY CLOSED MARRIAGE

Darwin (1876) began his book on the effects of selfing by recalling the conclusion from *Orchids* that Nature 'abhors perpetual self-fertilization', but he now emphasized the importance of the qualification 'perpetual': selfing might be beneficial under circumstances of mate limitation, but some crossing would eventually be required. To illustrate his point, he cited plants with 'cleistogamic' flowers, and later devoted an entire chapter to them in his book *The different forms of flowers* (Darwin 1877).

Cleistogamy, literally meaning 'a closed marriage', refers to plants that produce flowers in which

self-fertilization occurs within the closed bud (reviewed in Culley & Klooster 2007). The closed flowers are small, they produce very little pollen and no investment is made towards pollinator attraction or reward; they thus represent a superlatively efficient mode of self-fertilization. Significantly for Darwin, however, individuals of cleistogamous species also tend to produce open 'chasmogamous' flowers adapted to fertilization by outcrossing. Species with complete cleistogamy, i.e. in which no open flowers had ever been reported, were known to Darwin, and a recent review documents some 70 cases (Culley & Klooster 2007). However, we might join Darwin in his scepticism about claims of complete cleistogamy until it can be shown that a given species has no recent history of recombination. It would thus be worthwhile seeking evidence for recombination (see Wright *et al.* 2008) in the genomes of species in which open flowers have not been observed.

4. A STRICTLY CLOSED MARRIAGE AFTER ALL?

The term 'androdioecy' denotes the cooccurrence of males and hermaphrodites in a population. This sexual system is exceedingly rare (Darwin 1877; Charlesworth 1984), yet it has evolved a number of times independently in both plants and animals (Pannell 2002; Mackiewicz *et al.* 2006; Weeks *et al.* 2006). Male frequencies in androdioecious species typically vary considerably, and individual populations often lack males altogether. This pattern may be explained by a model of a metapopulation in which populations are initially colonized by hermaphrodites, which self-fertilize to produce hermaphrodite-only populations, and later joined by males when mating opportunities improve with population growth (Pannell 1997). The metapopulation model is a plausible explanation for both animal and plant examples of androdioecy (Pannell 2002), but androdioecious plants and animals differ in the mating behaviour of hermaphrodites.

In androdioecious plant species, hermaphrodites can self-fertilize, but they can also mate with both males and with each other, and the relative rates and fitness consequences of these three different mating events largely determine the proportion of males that can be maintained by selection (Charlesworth 1984). By contrast, the hermaphrodites of androdioecious animals, such as several species of the rhabditid nematodes (Kiontke *et al.* 2004), the clam shrimps (Sassaman & Weeks 1993) or the killifish (Mackiewicz *et al.* 2006), are unable to mate with each other; if they cannot cross with males, they must self-fertilize to reproduce. In these cases, males are maintained at a frequency that depends on the probability of male hermaphrodite encounters and the relative fitness of selfed versus outcrossed progeny (Otto *et al.* 1993; Pannell 2008).

In contrast with the situation in plants, the reproductive system of androdioecious animals thus implies that hermaphrodites in populations that lack males are condemned to perpetual self-fertilization. In this sense, a maleless population of an androdioecious animal

species is similar to a completely cleistogamous plant population. Extending this analogy, males in an androdioecious animal population might thus loosely be viewed as the open flowers of cleistogamous plants; only by virtue of the presence of males or open flowers is outcrossing and effective recombination possible.

It is not known how long the closed marriages of maleless populations of clam shrimps can persist, but males are completely unknown in some species. It is of course possible that males do indeed occur in these species but have not yet been found—just as Darwin suspected the occurrence of open flowers in putatively completely cleistogamous species—and maleless populations are common in species in which males do occur elsewhere. In these latter cases, it seems plausible that males might be maintained both by Darwinian selection within populations, as predicted by simple evolutionary models (Otto *et al.* 1993; Pannell 2008), and by a component of selection among groups. Species that have truly lost their males are perhaps on a one-way street to extinction, so that males persist at a higher taxonomic level partly as a result of the differential extinction of lineages. It would therefore be interesting to establish whether maleless populations of clam shrimps do indeed suffer increased extinction rates, and whether species in which males have never been found show any evidence of genome degeneration. These ideas remain untested for clam shrimps, but Loewe & Cutter (2008) have recently predicted that perpetual pure selfing in the androdioecious nematode *Caenorhabditis elegans* would lead to rapid lineage extinction.

Androdioecy in rhabditid nematodes such as *C. elegans* is similar to that in clam shrimps and killifish in that hermaphrodites cannot cross in the absence of males, but it is different in that males are generated from time to time as a result of the non-disjunction of the sex chromosome (Cutter *et al.* 2003). Although the occasional regeneration of males in *C. elegans* is probably not maintained by natural selection *per se* (Chasnov & Chow 2002; Cutter *et al.* 2003), it is possible that its (perhaps non-adaptive) occurrence has nevertheless contributed to lineage persistence. In *Eulimnadia* clam shrimps, outcrossing is potentially regained in a maleless population after the immigration of males, but this seems less possible in *C. elegans* because of the strong outbreeding depression that results from interpopulation mating (Dolgin *et al.* 2007). The sex determination system of *C. elegans* might thus be critical in leaving the door to an otherwise strictly closed marriage slightly ajar and in thus avoiding the ultimate fate of a commitment to perpetual self-fertilization.

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