

with the moth *Manduca* have shown that axons from one male antenna, entering an otherwise female brain, are sufficient to completely masculinize her behavior so that she flies towards another female releasing sex pheromone. If it were possible to swap the antennal imaginal discs of larval male fruit flies for female imaginal discs, what would happen to the connectivity of the genetically male aDNs? Would the scent of flies at communal oviposition sites attract males or induce courtship singing?

‘Same planet, different worlds’: as usual, Larson hit the nail on the head.

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Plant sex: Best to be bisexual when mates are scarce

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In flowering plants, transitions from bisexuality to unisexuality occur often, and have been considered irreversible. A new experimental evolution study demonstrates that this is unlikely to be true — ‘leaky’ sex expression can promote reversions to hermaphroditism when mates are scarce.

Most flowering plants produce female and male gametes and are thus hermaphrodites. But separate sexes — dioecy — have originated among nearly half of angiosperm families, although dioecious species are still rare, comprising only 5–6% of all species^{1,2}. Darwin was perplexed by the origin of dioecy in angiosperms, given their immobility and need for agents of gamete transfer for cross-fertilization (e.g., animals and wind). However, we now know that a variety of ecological and genetic factors favour the spread of unisexual plants in hermaphroditic populations, leading to dioecy³. The evolution of dioecy has been considered an example of irreversible evolution or Dollo’s law: the inability to re-acquire a recent ancestral state⁴. Indeed, the transition was characterized as an evolutionary ‘dead end’ because comparative data indicated that dioecious lineages were short-lived with

low diversification rates⁵. Theoretical models also suggested that dioecy can be disadvantaged compared with hermaphroditism under various ecological contexts⁶, potentially leading to extinction. But more recent studies have cast doubt on the dead-end scenario by demonstrating that dioecy does not generally retard diversification rates⁷, transitions from hermaphroditism to dioecy are not substantially more frequent than in the reverse direction⁸, and the molecular footprint of reversions from dioecy to hermaphroditism have been identified at the species level⁹. These results raise the question — what mechanisms promote reversions to hermaphroditism from dioecy? In a new study¹⁰, published in this issue of *Current Biology*, Guillaume Cossard and colleagues, working in John Pannell’s research group at the University of Lausanne, provide experimental evidence on how this can occur. Their findings may

also help explain the rarity of dioecy in flowering plants.

The research involved the European and Mediterranean wind-pollinated herb *Mercurialis annua*, which has become an outstanding study system for investigating the mechanisms driving sexual-system transitions. The species has remarkable sexual diversity (Figure 1), with populations that are either dioecious, monoecious (hermaphrodite plants with female and male flowers) or androdioecious (hermaphrodite and male plants). Pannell’s research group has exploited this variation to address how and why one sexual system evolves from another, and show that metapopulation dynamics and variation in plant density play key roles¹¹. Unlike most species with dioecy, *M. annua* is a prolific colonizer of disturbed environments, with a short annual life cycle — therefore, experimental populations can easily be allowed to evolve by growing successive

generations and following changes in phenotypic traits through time, including fitness components such as sex allocation¹².

The authors investigated the intriguing possibility of whether hermaphroditism might evolve in experimental populations composed of only a single sex. This question might at first seem improbable, but a relatively common and often unappreciated feature of dioecious plants — sex inconstancy — could potentially enable this process to occur. Sex inconstancy is the occurrence of a small minority of individuals in dioecious populations that produce gametes of the opposite sex; males producing a few ovules and females producing some pollen. This ‘leakiness’ in sex expression has both genetic and environmental components but functionally has the effect of rendering unisexual plants potentially capable of self-fertilization.

The evolutionary significance of sex inconstancy was first recognized by David Lloyd in his classic studies of the New Zealand genus *Leptinella*¹³, in which monoecy has evolved from dioecy. But efforts to demonstrate experimentally that sex inconstancy plays a role in promoting the breakdown of dioecy have not been attempted until now. Pannell’s group reasoned that because dioecious populations of *M. annua* have low levels of sex inconstancy, this could provide the standing genetic variation in gender to allow selection for the spread and perhaps fixation of hermaphroditism under the appropriate demographic conditions. Their earlier work¹¹ indicated that in *M. annua* dioecy is the ancestral sexual system, with monoecy and androdioecy as derived conditions (Figure 1).

In their new study, Cossard and colleagues established physically separated, replicated garden populations of two types: female-only populations, and those with a 1:1 ratio of females and males typical of wild populations (Figure 2A,B, respectively). The experimental populations were followed annually for four generations, with seed collected each generation and sowed the next year. They found that in female-only populations, there was a striking increase in the frequency of

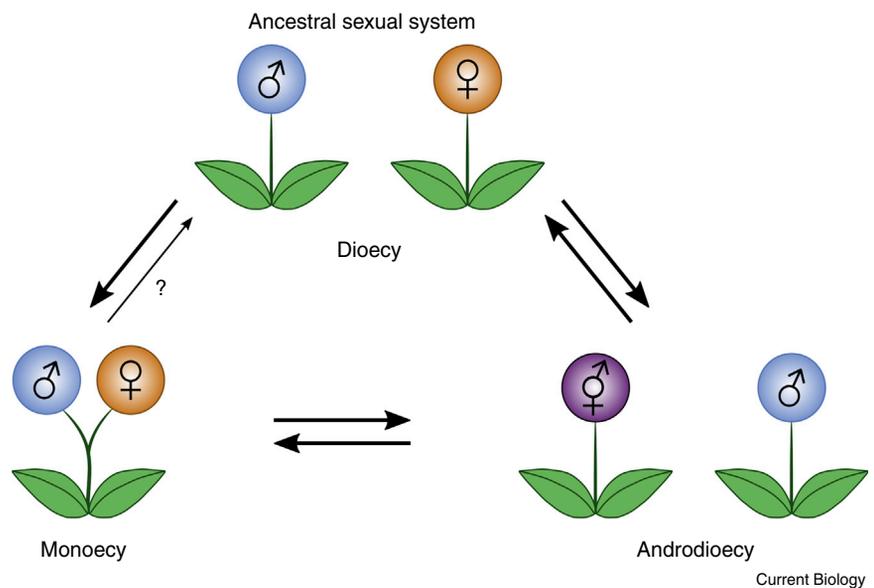


Figure 1. Evolutionary lability of sexual systems in *Mercurialis annua*.

Illustrated are the three primary sexual systems — dioecy, monoecy and androdioecy — with arrows indicating transitions among them. These transitions are largely governed by metapopulation dynamics, changes in plant density and reproductive assurance following colonization¹¹. Direct transitions from monoecy to dioecy have as yet not been observed in *M. annua*.

females producing male flowers (Figure 2C), and in the number of male flowers produced by these individuals. Specifically, in the final generation, 69% of the plants in the female-only populations produced male flowers, and there was a 23-fold increase in average male flower production. This phenotypic masculinization of females was not evident in experimental populations with equal sex ratios.

The most plausible hypothesis to explain the rapid increase in sex inconstancy in female-only populations is that females with male flowers had higher fitness than their strictly unisexual counterparts, and were therefore favoured by natural selection. However, the authors also evaluated the possibility that phenotypic plasticity may have contributed to the changes they observed. To distinguish between these possibilities, they grew plants from both treatments (female-only and equal sexes), and all four generations, in a single common garden. They found similar levels of sex-inconstancy, as observed across years in the separate gardens, in females from female-only populations. Although plasticity plays some role in male flower production in females, evolution driven by natural selection appears to be the primary

determinant of the large changes in male sex allocation they observed. The measured evolutionary rates reported in this study are among the highest recorded for any group of organisms¹⁴.

Mate limitation is a major factor explaining the predominance of hermaphroditism in plants and other sessile organisms across the tree of life¹⁵. But the benefit of hermaphroditism when mating partners are sparse is particularly large if plants are able to self-fertilize. To investigate whether sex inconstancy facilitated selfing, Cossard and colleagues used genetic markers to measure mating patterns in dioecious and female-only populations in their common garden experiment. As expected, they detected no selfing in females from dioecious populations, but selfing rates as high as 22% were evident in plants from female-only populations. Paternity analysis demonstrated that sex-inconstant females could also sire offspring when in competition with males from dioecious populations. This newly acquired mating flexibility probably facilitated the evolution of monoecy from dioecy in *M. annua* (Figure 1).

The results of this study on *M. annua* elegantly demonstrate that, in female

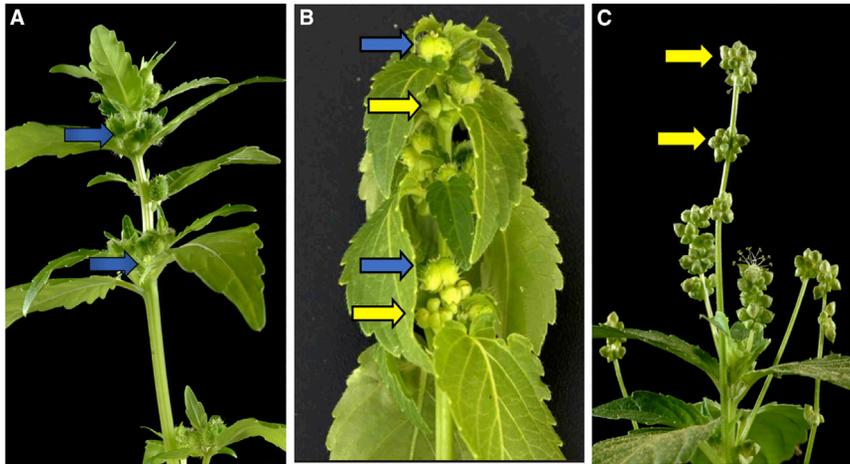


Figure 2. The three sex phenotypes of *Mercurialis annua* in the experimental evolution study. Blue and yellow arrows point to female flowers (or fruits) and male flowers, respectively. (A) Female with flowers and fruit. (B) Sex-inconstant female with both female and male flowers. (C) Male with male flowers. Note the different positions of male flowers in sex inconstant females, where they are largely sessile, and males where they are borne on erect peduncles. (Images courtesy of John Pannell.)

arrays where plants were deprived of male mating partners, selection for reproductive assurance and a capacity to sire outcross progeny favoured females that produced pollen. The enhancement of male flower production probably resulted from classical Fisherian sex-ratio selection. Negative frequency-dependent selection favoured increased allocation to male flowers providing more mating opportunities, especially during earlier generations of the experiment when levels of sex inconstancy were at their lowest.

What lessons and further questions arise from this study that can inform future research on transitions in plant sexual systems? Although manipulative experiments are frequently used in plant reproductive ecology, the application of experimental evolution approaches is relatively novel. Earlier studies demonstrated rapid evolution of selfing traits under pollen limitation in monkey flower (*Mimulus*)¹⁶, and divergent selection on floral traits in rapid cycling *Brassica* visited by bumble bees versus hoverflies¹⁷. Clearly, there is considerable scope for using the power of this method to study real time evolution of reproductive traits in species with short life cycles. An intriguing question emerging from this study concerns the mechanisms governing male reproductive structures in females. The

secondary sex characters responsible for sex function in males of *M. annua* reside on the Y chromosome, but females lack this chromosome, and therefore the genes responsible for male traits must be elsewhere in the genome. Identifying where these genes occur and how they function would be valuable to determine whether male sexual structures can arise by different genetic and developmental pathways, as occurs for male traits involved with the evolution of selfing in *Eichhornia paniculata*¹⁸.

Finally, this study highlights two important general features of sexual-system evolution — the important role that mate limitation can play in the evolution of plant mating strategies, including among the diverse sexual systems in *M. annua*. And the strength of Fisherian sex-ratio selection, which can result in rapid changes in sex allocation when mating opportunities change. The results of this investigation also provide a possible explanation of why dioecy is uncommon in angiosperms — it often evolves into other sexual systems.

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