Commentary

Rapid adaptation of *Viola arvensis* to pollinator declines

Most flowering plants depend on animal vectors for successful pollination. How plant populations respond when pollinators decline in abundance, or are completely lost from a habitat, is a key question for conservationists, ecologists, and evolutionary biologists. The demographic consequences of pollinator loss are an immediate conservation concern given global climate change. Many pollinating species such as bees are declining severely (Goulson *et al.*, 2015). The loss of these species, itself a biodiversity crisis, will have cascading effects on plant communities and their contribution to ecosystem functions. Of course, fluctuations and localized extinctions of pollinators are not a new phenomenon and have likely been occurring throughout the evolutionary history of flowering plants. The failure of pollinator service may be a driving force behind the recurrent evolution of the 'selfing syndrome' across flowering plants. The selfing syndrome is a collection of morphological and life history changes that occur within lineages that transition from outcrossing to selfing (Ornduff, 1969). Typical changes are reductions in flower size, in the physical separation of male and female reproductive organs, and the ratio of investment in pollen relative to ovules.

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The selfing syndrome is one of the most famous examples of parallel evolution because similar changes have occurred independently in many unrelated groups of plants. Yet, despite many years of study, questions remain. For example, do selfing syndrome traits change concurrently with the change in the rate of self-fertilization within a population or after the shift to predominant selfing has occurred (driven by selection to increase the efficiency of seed production)? Do the various traits constituting the syndrome evolve simultaneously or sequentially? Can the selfing syndrome be 'constructed' from genetic variation that is present within the ancestral outcrossing population or do selfing lineages acquire many of their distinctive features by fixing new mutations after they become predominantly selfing? The paper by Acoca-Pidolle *et al.* (2024, 717–726) published in this issue of *New Phytologist* provides fresh insight into these questions. Their study characterizes evolutionary change in selfing syndrome traits, as it is currently occurring, in wild populations of the field pansy, *Viola arvensis.*

This article is a Commentary on Acoca-Pidolle *et al.* (2024), **242**: 717–726.

Acoca-Pidolle *et al.* describe a multi-part study of contemporary evolution within four natural populations of *V. arvensis* that have experienced reduced pollinator service. The populations surround Paris, France, where urbanization is thought to have reduced the abundance of pollinators. Acoca-Pidolle *et al.* first use genotyping at molecular markers (microsatellite loci) to show that plants are substantially more homozygous, relative to Hardy–Weinberg expectations, now than they were 20 years ago. The estimated rate of selfing has increased 27% (on average across populations) over this time interval. Next, to determine whether traits have evolved to accompany this change in selfing rate, Acoca-Pidolle *et al.* perform a 'resurrection experiment'. They grew plants from seeds collected 20 years ago in a common garden environment with their descendants sampled in 2021. In this common environment, the descendant populations exhibit consistent phenotypic differences from their ancestors. They observed trait evolution toward smaller corollas, reduced nectar production, and reduced attractiveness to bumblebees, with parallel shifts in the same traits across all four locations that were monitored. Trait evolution was clearly concurrent with mating system change. The estimated change in selfing rate (from an average of 0.54 in the ancestral populations to 0.82 in their descendants) was substantial but incremental. Major aspects of the syndrome are emerging in *V. arvensis* even though these populations are yet a long way from fully selfing.

The resurrection method is being applied to an increasing number of species (Weider *et al.*, 2018). In part, this reflects opportunity – more comprehensive population sampling combined with improved seed storage protocols make these studies possible. Perhaps more importantly, botanists (and even some zoologists) have come to appreciate the dynamic scale of evolution, of how much measurable change routinely occurs on ecological timescales. Resurrection experiments reveal these changes directly, but like all methods, are subject to caveats. Franks *et al.* (2018) recently reviewed resurrection methodology and suggest guidelines for experimental design. The study of Acoca-Pidolle *et al.* illustrates several important design principles. For example, seed age can affect plant growth and development apart from any genetic evolution distinguishing ancestral and descendant populations. Acoca-Pidolle *et al.* genotyped plants that were produced (as seed) in the natural populations in order to estimate selfing rates. However, their phenotypic measurements were taken on plants derived from a 'refresher generation', which serves to erase any effects owing to differences in seed age or maternal environment. A second concern is that genetic differences between ancestral and descendant populations may not reflect adaptation. A major population crash, as might easily result from pollinator failure, could generate nonadaptive genetic differentiation via genetic drift. The inclusion of micro-satellite genotyping data provides a test for the effect of potential bottlenecks. Three of the four *V. arvensis* showed no decline in allelic richness from ancestor to descendant. Perhaps more obviously, all four of the 'replicate' populations showed parallel changes in morphology, consistent with evolution by natural selection but not drift.

The direct effect of inbreeding is also a key consideration when mating system differs between ancestral and descendant populations. 'Inbreeding depression' is the well-known tendency for fitness measures to decline with inbreeding (Darwin, 1876). Despite the common assumption of additivity, quantitative traits such as flower size also routinely change with inbreeding (e.g. Shaw *et al.*, 1998). The concern is that if two populations differ in their average level of inbreeding (typically measured by F, the inbreeding coefficient), they will differ in trait values even if there has been no adaptation (in terms of allele frequency change from ancestral to descendant population). Acoca-Pidolle *et al.* address this by estimating both the effect of inbreeding on traits and the inbreeding coefficient in each population of measured plants. Combining these two sources of information, they find that the estimated direct effect of inbreeding on traits is much smaller than the evident differences between ancestral and descendant means for mating system traits. This implies a genuine response to natural selection.

The trait changes documented by Acoca-Pidolle *et al.* are, in a sense, consistent with prior expectations. Populations with higher rates of selfing are shifting trait values in a way predicted to be favorable for more effective/efficient selfing. However, the evolution of increased selfing should not be taken as an inevitable response to anthropogenic changes in the environment. Like demographic effects, evolutionary responses to contemporary environmental changes are likely to be idiosyncratic. For example, in another recent resurrection study, Bishop *et al.* (2023) found that some local populations of morning glories have evolved increased flower size and investment in floral rewards over a 9-yr interval. The Acoca-Pidolle *et al.* study is instructive not only because the result is unusually clear (parallel phenotypic changes across multiple extant populations that reiterate a broad phylogenetic pattern), but also because it illustrates how to synthesize phenotypic and molecular data. Genotyping was essential to confirm that the putative agent of selection (reduced pollinator abundance) was having the predicted effect (increased self-fertilization) and also to deflect alternative explanations (genetic drift owing to severe population bottlenecks). Given that allele frequencies as well as trait means are changing rapidly in natural populations (Brown & Koenig, 2022; Kreiner *et al.*, 2022), an important goal for future studies will be to integrate the genomic sequencing into the phenotypic resurrection experiment.

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