

VIEWPOINT: PART OF A SPECIAL ISSUE ON PLANT MATING SYSTEMS

New perspectives on the evolution of plant mating systems

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• **Background** The remarkable diversity of mating patterns and sexual systems in flowering plants has fascinated evolutionary biologists for more than a century. Enduring questions about this topic include why sexual polymorphisms have evolved independently in over 100 plant families, and why proportions of self- and cross-fertilization often vary dramatically within and among populations. Important new insights concerning the evolutionary dynamics of plant mating systems have built upon a strong foundation of theoretical models and innovative field and laboratory experiments. However, as the pace of advancement in this field has accelerated, it has become increasingly difficult for researchers to follow developments outside their primary area of research expertise.

• **Scope** In this Viewpoint paper we highlight three important themes that span and integrate different subdisciplines: the changes in morphology, phenology, and physiology that accompany the transition to selfing; the evolutionary consequences of pollen pool diversity in flowering plants; and the evolutionary dynamics of sexual polymorphisms. We also highlight recent developments in molecular techniques that will facilitate more efficient and cost-effective study of mating patterns in large natural populations, research on the dynamics of pollen transport, and investigations on the genetic basis of sexual polymorphisms. This Viewpoint also serves as the introduction to a Special Issue on the Evolution of Plant Mating Systems. The 15 papers in this special issue provide inspiring examples of recent discoveries, and glimpses of exciting developments yet to come.

Key words: Biparental inbreeding, heterostyly, mate diversity, mixed-mating system, next generation sequencing, outcrossing, paternity, phenotypic plasticity, plant breeding systems, pollination, self-fertilization, sexual polymorphism.

INTRODUCTION

Biologists have long been fascinated by the remarkable diversity of mating patterns and sexual systems in flowering plants. Why, for example, do proportions of self- and cross-fertilization often vary dramatically among populations and even among individuals of the same species? Why have sexual polymorphisms evolved over 100 times, yet are found in only a small minority of plant species? Do plants with high rates of self-fertilization have reduced outcross siring success? Why does the composition and diversity of mates vary markedly within and among populations? These are fundamental questions in evolutionary biology and have been the focus of a large body of productive mathematical modelling and innovative empirical study (Darwin, 1876, 1877; Fisher, 1941; Bateman, 1948; Lloyd, 1992; Schoen and Lloyd, 1992; Lloyd and Barrett, 1996; Dudash *et al.*, 1997; Pannell and Barrett, 1998; Griffin and Eckert, 2003; Teixeira and Bernasconi, 2007; Case and Ashman, 2009).

Research on the evolution of plant mating systems has flourished over the past decade (e.g. Kalisz *et al.*, 2004; Delph and Wolf, 2005; Goodwillie *et al.*, 2005; Harder and Barrett, 2006; Barrett, 2010; Eckert *et al.*, 2010; Bodbyl Roels and Kelly,

2011). However, as the pace of advancement in evolutionary biology and population genetics has accelerated, it has become increasingly difficult for researchers to continue to follow developments outside their primary area of research expertise. Therefore, a major goal of this Special Issue on Plant Mating is to showcase research that spans and integrates different subdisciplines.

Here we highlight some of the recurring themes of papers in the Special Issue. These include: the changes in morphology, phenology and physiology that accompany the transition to selfing; the evolutionary consequences of pollen pool diversity in flowering plants; and the evolutionary dynamics of sexual polymorphisms. We also highlight recent developments in molecular techniques that will facilitate more efficient and cost-effective study of mating patterns in large natural populations, research on the dynamics of pollen transport, and investigations on the genetic basis of sexual polymorphisms.

UNSOLVED MYSTERIES IN THE TRANSITION TO SELF-FERTILIZATION

Some of the most confounding puzzles in plant mating system evolution concern the suite of changes in morphology,

phenology and physiology that typically accompany the transition to selfing (Darwin, 1876; Ornduff, 1969; Wyatt, 1988; Vallejo Marín and Barrett, 2009; Mazer *et al.*, 2010; Sicard and Lenhard, 2011; Ivey and Carr, 2012, this issue; Kalisz *et al.*, 2012, this issue). For example, do traits such as small flower size, reduced separation of anthers and stigma (Fig. 1), and decreased investment in male allocation evolve simultaneously with self-fertility, or sequentially? If sequentially, is there a predictable order? Surprisingly little is known about such questions, despite the profound biological importance of this common evolutionary transition (Stebbins, 1974; Hamrick and Godt, 1989; Goldberg *et al.*, 2010). Although much remains to be learned, recent research has provided valuable insights. For example, changes in life history, floral display and floral morphology that accompany transitions to selfing have been identified through comparative analyses within lineages that have both selfing and outcrossing species (Barrett *et al.*, 1996; Weller and Sakai, 1999; Ferrero *et al.*, 2012, this issue; Kalisz *et al.*, 2012, this issue). Furthermore, effects of key floral traits on variation in selfing rates have been measured in several species (Holtsford and Ellstrand, 1992; Karron *et al.*, 1997; van Kleunen and Ritland, 2004; Takebayashi *et al.*, 2006; Kennedy and Elle, 2008; Eckert *et al.*, 2009; Dart *et al.*, 2012, this issue; Kalisz *et al.*, 2012, this issue). In addition, adaptive trait change (a reduction in herkogamy) has been directly observed in a five-generation greenhouse experiment that excluded pollinators from *Mimulus guttatus* (Bodbyl Roels and Kelly, 2011). Studies such as these have begun to provide clues about the transition to selfing, but the sequence of trait transitions and the evolutionary mechanisms driving these transitions have yet to be demonstrated for any species.

Obtaining estimates of the strength of selection on candidate mating system traits is an important first step for clarifying the mechanisms involved in their evolution (Fenster and Ritland, 1994; van Kleunen and Ritland, 2004). Such fitness estimates need to include both male and female function, as well as fitness costs of selfing (*sensu* Lau *et al.*, 2008; Busch and Delph, 2012, this issue; Karron and Mitchell, 2012, this issue). Phenotypic manipulations of traits can help reveal how they influence fitness, as well as their contributions to different modes of selfing (Schoen and Lloyd, 1992; Campbell



FIG. 1 *Gilia achilleifolia* exhibits striking within-population variation in the degree of herkogamy, which is associated with variation in selfing rate. Individuals with high herkogamy self less than individuals with low herkogamy. Photograph by Naoki Takebayashi.

et al., 1996; Eckert, 2000; Fetscher, 2001). More generally, well-designed experiments can directly test mechanistic hypotheses about trait evolution, such as the extent to which mating system traits evolve through direct selection, as opposed to correlated evolution with other adaptive traits (Fenster and Ritland, 1994; Barrett *et al.*, 1996; Mazer *et al.*, 2004; Ivey and Carr, 2012, this issue).

A second area ripe for investigation concerns how environmental variation may facilitate mating system transitions (Lloyd, 1992; Ashman, 2006). Several theoretical and empirical studies have addressed the influence of pollinators on the evolution of selfing (Holsinger *et al.*, 1984; Lloyd, 1992; Barrett and Harder, 1996; Goodwillie *et al.*, 2005; Morgan *et al.*, 2005; Karron *et al.*, 2009; Karron and Mitchell, 2012, this issue). However, other environmental influences also appear to be important and warrant further study. For example, interactions with natural enemies or variation in the abiotic environment can alter the expression of inbreeding depression (Ivey *et al.*, 2004; Armbruster and Reed, 2005; Cheptou and Donohue, 2011), male fertility (Quesada *et al.*, 1995; Mutikainen and Delph, 1996; Caruso *et al.*, 2005, Herlihy and Delph, 2009), selfing rates (Ivey and Carr, 2005; Steets *et al.*, 2006), and other mating system features (Ashman, 2006; Steets *et al.*, 2007; Eckert *et al.*, 2010). These results suggest that a diversity of environmental factors may also influence transitions in the mating system.

A third promising area for research concerns the role of environmental variation in mating system evolution through phenotypic plasticity (e.g. Levin, 2010, 2012, this issue; Murren and Dudash, 2012, this issue). For example, plastic variation in traits correlated with selfing rate may help explain a long-standing puzzle in mating system biology: the evolutionary stability of mixed mating systems (Waller, 1980; Elle and Hare, 2002; Goodwillie *et al.*, 2005; Ivey and Carr, 2005; Steets *et al.*, 2006). In addition, plastic responses in mating system traits can facilitate colonization or adaptation to marginal habitats, which can pave the way for mating system transitions in stressful or novel habitats (Holtsford and Ellstrand, 1992; Levin, 2010; Cheptou, 2012, this issue; Ivey and Carr, 2012, this issue; Levin, 2012, this issue). Trait plasticity, however, need not inevitably lead to mating system transitions. For example, herkogamy can show marked plasticity (Fig. 2; Elle and Hare, 2002; Ivey and Carr, 2005; Brock and Weinig, 2007; Vallejo Marín and Barrett, 2009; de Waal *et al.*, 2012, this issue), which may make the transition to selfing likely in some environments (e.g. where anther–stigma separation is lower) but favour outcrossing in others (Lloyd, 1992; Morgan *et al.*, 2005). As with sex-differential phenotypic plasticity (see below), little is known about the heritability of plasticity in traits affecting selfing rates (Vallejo Marín and Barrett, 2009; Ivey and Carr, 2012, this issue). Therefore the extent to which plasticity in mating system traits might be an adaptive response to fluctuating environments deserves additional study (Levin, 2012, this issue).

Transitions to selfing undoubtedly occur through diverse pathways for different lineages. Nonetheless, dissection of the evolutionary mechanisms leading to these mating system traits, and their environmental and genetic influences, will

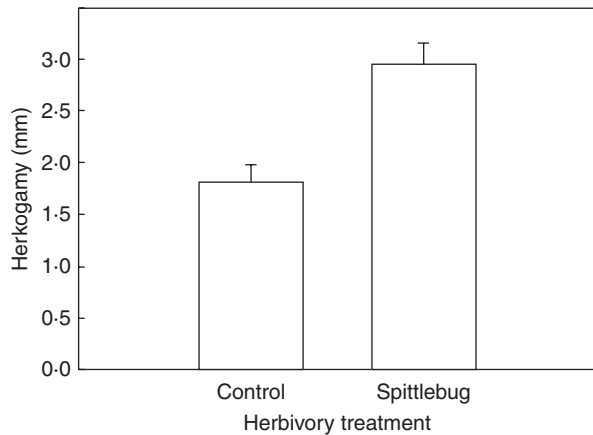


FIG. 2 Herbivory by spittlebugs influences mating system traits in *Mimulus guttatus*. Plants in field populations that were experimentally exposed to spittlebug herbivory had 63% greater herkogamy than control plants that did not receive herbivory. Redrawn from Ivey and Carr (2005).

help unravel the secrets behind this common feature of hermaphroditic reproduction.

EXPANDING THE CONCEPT OF MATING SYSTEM IN SELF-COMPATIBLE HERMAPHRODITES

When a pollinator visits a hermaphroditic flower it often deposits a mixture of pollen from several different sources, including self pollen, pollen from close relatives, and pollen from one or more unrelated donors (Fig. 3; Waser, 1993; Karron *et al.*, 2006). Although this diverse pollen pool is often dichotomized as ‘self vs. outcross’, we believe that a deeper understanding of the diversity of outcross pollen, the factors influencing these mating patterns, and the fitness consequences of these mating events is needed.

One important component of a diverse mating pool is crossing between close relatives (biparental inbreeding), which may be especially likely in populations that have substantial genetic structure (Waller, 1993; Griffin and Eckert, 2003). When pollinators move short distances and pollen carryover is limited, a large fraction of the pollen deposited on a stigma might come

from close relatives (Mitchell *et al.*, 2009). These inbred (but non-self) matings can substantially reduce heterozygosity and, if inbreeding depression is present, lower offspring fitness (Nason and Ellstrand, 1995). The reduction in fitness due to biparental inbreeding is thought to influence the evolution of mating systems by changing the relative costs and benefits of selfing vs. outcrossing (Uyenoyama, 1986; Yahara, 1992; Waller, 1993). For example, when deleterious recessive alleles are expressed through biparental inbreeding, the fitness difference between self- and outcross progeny is reduced (Waller, 1993).

Surprisingly, the extent of biparental inbreeding (b) and the fitness consequences of biparental inbreeding depression have rarely been quantified in natural populations. Most attempts to quantify biparental inbreeding using marker genes have compared estimates of selfing based on single marker genes (s_s) with estimates based on multiple marker genes (s_m ; Brown, 1990). Although theory suggests that $s_s - s_m$ will give an estimate of b (Shaw *et al.*, 1981), this parameter is usually substantially underestimated due to an insufficient number of marker loci (Leclerc-Potvin and Ritland, 1994; Ritland, 2002). A more powerful approach is to compare the selfing rate of experimental populations that have been randomly rearranged to break up genetic structure with the selfing rate of paired control populations where plants were returned to their original locations (Kelly and Willis, 2002). Using this approach, a study of *Aquilegia canadensis* demonstrated that nearly 30% of all matings were between close relatives (Griffin and Eckert, 2003). Given the high level of inbreeding depression in *A. canadensis*, full sib mating is likely to have a dramatic effect on fitness (Herlihy and Eckert, 2004). More work is needed to thoroughly assess the extent of biparental inbreeding in natural populations, to quantify its fitness consequences, and to determine the ecological conditions that tend to promote biparental inbreeding.

The dichotomy of selfing versus outcrossing also masks important differences in the diversity of donors siring seeds within fruits (Bernasconi, 2003; Bernasconi *et al.*, 2004). Theory suggests that clutches sired by multiple fathers will have greater variation in progeny performance, increasing the likelihood that favourable combinations of maternal and paternal genes will be generated (Falconer, 1981; Yasui, 1998; Whittingham and Dunn, 2006). An increase in mate

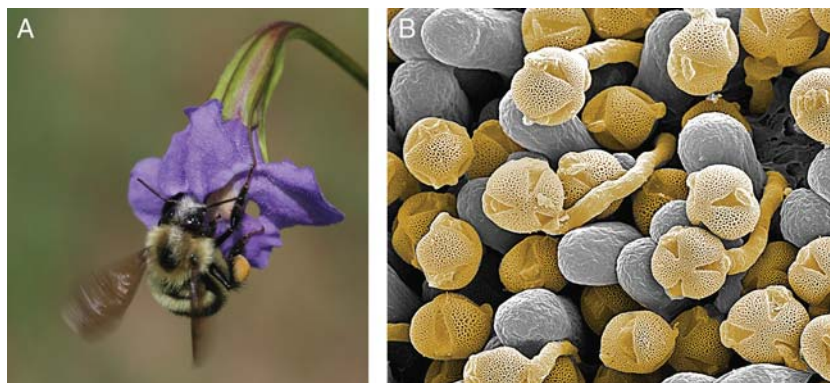


FIG. 3 (A) Bumble bees typically deposit a mixture of pollen from multiple pollen donors onto the stigma of a receptive *Mimulus ringens* flower. (B) Pollen grains (coloured gold) germinating on a *Mimulus ringens* stigma. Photographs by Jeff Karron, Randall Mitchell and John Bell.

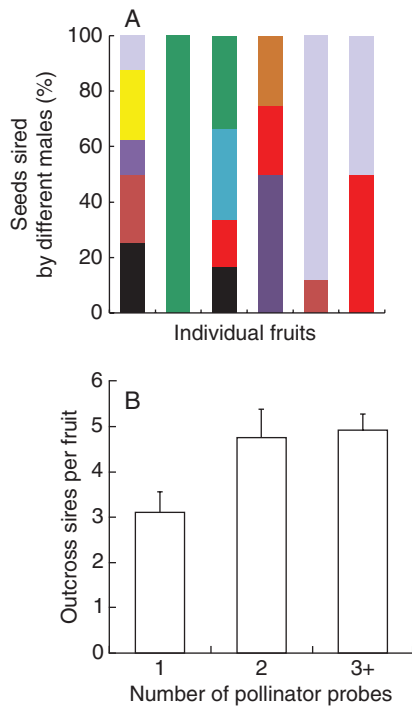


FIG. 4 (A) Outcross sire profile for six *Mimulus ringens* flowers that received a single visit. Colours indicate sires of the seeds. (B) An increase in the number of visits to individual *Mimulus ringens* flowers increases sires per fruit ($P < 0.01$). Modified from Karron *et al.* (2006).

diversity within fruits also decreases genetic relatedness among siblings (Ritland, 1989), potentially intensifying competition among maternal half-sibs (Karron and Marshall, 1990, 1993; Bernasconi, 2003).

Researchers studying vertebrates have suggested that females may engage in multiple mating (polyandry) to 'hedge' their bet that their initial mate or mates will provide a sufficient number of high-quality gametes (Double and Cockburn, 2000; Fox and Rauter, 2003; Whittingham *et al.*, 2010). Could such a strategy occur in flowering plants? In fruits of *Mimulus ringens*, outcross seeds are sired by one to eight pollen donors (Mitchell *et al.*, 2005). Karron *et al.* (2006) demonstrated that sequential pollinator probes to individual flowers significantly enhance mate diversity within fruits (Fig. 4). If plants can regulate the timing of stigmatic receptivity, stigma closure or floral longevity, they could potentially influence mate diversity within fruits (Bernasconi, 2003; Lankinen and Madjidian, 2011). More research is needed to understand how mate diversity varies within and among populations, the factors regulating mate diversity, and the fitness consequences of variation in mate diversity in natural populations.

EVOLUTIONARY DYNAMICS OF SEXUAL POLYMORPHISMS

Studies of sexual polymorphisms, such as separate sexes and heterostyly, can provide important insights into the evolution of floral form and function. Gender and stelar polymorphisms

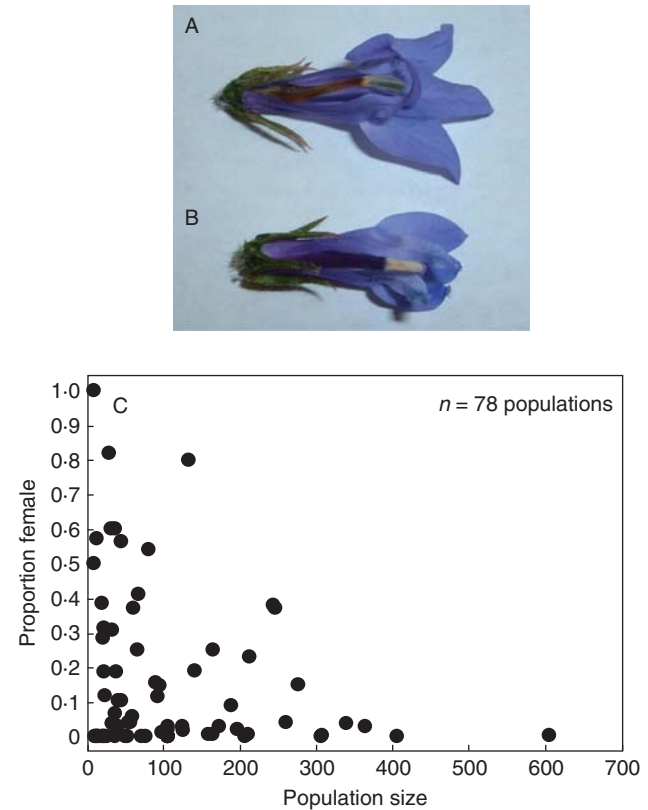


FIG. 5 Gyndioecious *Lobelia siphilitica* populations contain (A) hermaphrodites and (B) females. (C) Relationship between population size (number of plants) and sex ratio in natural populations of *L. siphilitica* in eastern North America. Data are from Caruso and Case (2007) and Proell (2009). Smaller populations exhibit a wider range of female frequencies. Photograph by Maia Bailey.

have each evolved multiple times across the Angiosperms, and ratios of sexual morphs often vary widely within and among species (Figs 5 and 6). Thus, these polymorphisms provide natural experiments for studying how variation in sex-organ morphology influences pollinator visitation, mating success and sex-specific fitness gain while holding other aspects of the species' biology more-or-less constant. By taking advantage of natural variation within and among populations, recent work has begun to evaluate the relative strength of two contrasting processes that can influence the distribution of sexual morphs within species: natural selection (including frequency-dependent selection) and stochastic processes (such as genetic drift, extinction-colonization and metapopulation dynamics; Pannell and Dorken, 2006; Barrett *et al.*, 2009; McCauley and Bailey, 2009).

Disentangling the outcomes of selection from the outcomes of stochastic processes is not an easy task because both processes can yield similar patterns. For example, sexual morph ratios within a population may reflect selection favouring one morph over another, but such a pattern may also result from stochastic changes in the spatial distribution of alleles that underlie or modify sexual phenotypes. Morph ratios are known to vary markedly when population size is small (Fig. 5; Caruso and Case, 2007; Brys *et al.*, 2008), when

populations have low levels of neutral genetic diversity (Cuevas *et al.*, 2006; Ness *et al.*, 2010; Li *et al.*, 2012, this issue), or when extinction–colonization dynamics are clearly present (Pannell and Dorken, 2006).

In order to fully evaluate the roles of selective and stochastic processes on sexual-system variation within species, it is important to determine the distribution of morph-determining alleles within and among populations (De Cauwer *et al.*, 2011). If morph determination has a simple genetic basis (such as a single nuclear locus), the sexual morphs themselves are reliable markers for those alleles. Thus, inferences from neutral genetic markers are effective for examining how stochastic processes affect morph ratios (e.g. Hodgins and Barrett, 2007; Ness *et al.*, 2010). However, in many species genetic control of sexual polymorphisms is complex or is poorly understood. This is especially true for some gynodioecious species (Delph *et al.*, 2007). In these latter cases, it may be unreasonable to assume that the distribution of neutral genetic markers clearly represents stochastic forces acting on morph-determining alleles. As described in more detail below, new technological innovations in DNA sequencing may soon help fast track progress in this area.



FIG. 6 Subdioecy in *Wurmbea dioica*. Populations contain three sex morphs (left-to-right): hermaphrodite, male and female. See Vaughton and Ramsey (2012). Photograph by Glenda Vaughton and Mike Ramsey.

The role of stochasticity is of particular interest for understanding why and how sexual systems evolve from one state to another (reviewed in Barrett, 2010). Although transitions must certainly involve deterministic forces, such as selection on morph-specific fitness, they may also be facilitated by chance events affecting morph ratios. Stochastic loss of style morphs from populations can alter mating dynamics in heterostylous species, and favour transitions between sexual-system states (Fig. 7; Barrett *et al.*, 2009). Population sex-ratio variation in some gynodioecious species may in fact be largely stochastic, driven by processes such as genetic drift, founder effects, and extinction–colonization dynamics (Bailey and Delph, 2007). However, the role of stochasticity in evolutionary transitions to and from separate sexes may depend strongly on the genetics of sex determination. Although solid empirical data are scarce, stochastic effects are predicted to matter more for species with cytonuclear sex determination compared to those with nuclear sex determination (McCauley and Bailey, 2009; Dufay and Pannell, 2010).

Just as stochastic forces may influence transitions between sexual system states, phenotypic plasticity in morph-determining traits may also play a critical evolutionary role. Of particular interest is whether sexual plasticity facilitates or constrains sexual-system evolution. Plasticity may facilitate sexual-system transitions such as (a) from combined to separate sexes (Dorken and Michard, 2008; Vaughton and Ramsey, 2012, this issue), and (b) from outcrossing to selfing (Vallejo-Marin and Barrett, 2009; Dart *et al.*, 2012, this issue; Levin, 2012, this issue). By contrast, gender plasticity may constrain transitions to full dioecy (Delph, 2003; Delph and Wolf, 2005; Ehlers and Batillon, 2007; Spigler and Ashman, 2012, this issue).

Whether or not plasticity facilitates or constrains transitions between sexual systems will depend on whether genes underlying plasticity also influence the expression of sexual polymorphisms. Without better information on the genetic basis of both plasticity and sex determination, this remains challenging to address. Studies such as Spigler and Ashman (2011) and Vaughton and Ramsey (2012, this issue) provide important insights into the consequences of sex-differential plasticity in two ways. First, they show that the probability, extent and cost of gender plasticity vary among sexual system states. In subdioecious populations, a lower proportion of hermaphrodites exhibit plasticity, their capacity to adjust their sex allocation is more limited, and plasticity is more costly compared to hermaphrodites in gynodioecious species (Vaughton and Ramsey, 2012, this issue). Second, these studies suggest genetic variation for sex-differential plasticity that should not

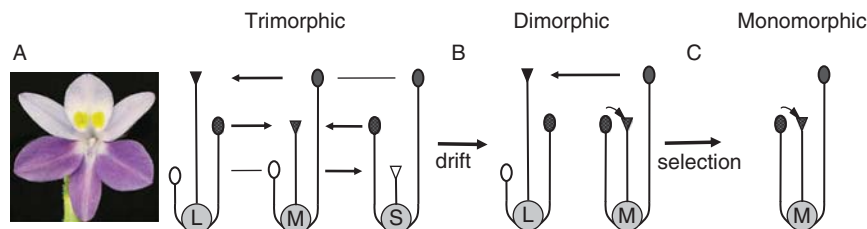


FIG. 7 Transition from outcrossing to selfing in (A) tristylous *Eichhornia paniculata* occurs in two steps: (B) genetic drift results in the loss of the S morph, (C) resulting in selection for reproductive assurance in the M morph. Redrawn from Barrett *et al.* (2009). Photograph by Spencer Barrett.

necessarily constrain selection for increased gender specialization. However, observed plasticity itself may reflect selection to stabilize intermediate sexual systems.

Much remains to be learned about how particular types of sex determination influence sexual-system transitions. Additional insights may be gained by assessing the variability of sexual systems among close relatives. Comparative studies of sexual-system evolution within clades reveal important patterns in traits associated with sexual systems. Phylogenetic studies also provide insights concerning the order in which these traits arise (e.g. *Alonso and Herrera, 2011; Ferrero et al., 2012, this issue*). However, we suggest that sexual-system studies within species should also be viewed in the broader context of the evolutionary lability of sexual systems within clades. Frequently-studied gynodioecious taxa include those that are closely related to dioecious species (e.g. *Fragaria, Silene, Wurmbea*), as well as taxa that have no documented dioecy either within the same genus (e.g. *Beta, Thymus, Daphne, Kallstroemia*) or the same family (e.g. *Geranium, Nemophila, Plantago*; <http://www.umsl.edu/~renners/Dioecy%20table%20www.pdf>). Gynodioecious species may have no close dioecious relatives because they have little propensity to evolve dioecy. Thus, categorizing species based on this propensity may help to identify traits that permit or facilitate the full transition to dioecy versus traits that may constrain it.

To illustrate this point, we have reviewed data presented by *Dufay and Billard (2012, this issue)*. In their paper, these authors assessed whether inbreeding avoidance is likely to play an important role in the maintenance of female plants, as commonly predicted for gynodioecious species (*Charlesworth, 1999*). *Dufay and Billard (2012, this issue, table 5)* identify seven out of 21 species in their survey where avoidance of inbreeding is likely to contribute to selection favouring females. However, if we look at those seven species based on the sexual systems of their relatives, all but two also show a phylogenetic association with dioecy (i.e. they have dioecious relatives). In contrast, most (11 of 14) of the species for which inbreeding avoidance was deemed unlikely to be important lack dioecious relatives. Thus, a more precise inference about the variable role of inbreeding avoidance in female advantage may be possible. Inbreeding avoidance may maintain females in gynodioecious species in clades where transitions to full dioecy have occurred. By contrast, inbreeding avoidance may be a less important component of female advantage in clades that have evolved gynodioecy but not dioecy.

AVENUES FOR FUTURE RESEARCH

The role of pollinators in the mating system

Studies of mating system patterns seldom incorporate the dynamics of the pollination process. Therefore mating system mechanisms are often divorced from mating system outcomes (*Harder and Barrett, 1996*). For example, few studies can directly link pollinator movements to the realized mating system of flowers visited by those pollinators (*Brunet and Sweet, 2006; Karron et al., 2004; 2009; Karron and Mitchell, 2012, this issue*). Yet a synthetic approach is critical to understanding

fundamental questions such as: how would the loss or decline of the primary pollinator of a species influence outcrossing rates, mate diversity and patterns of pollen-mediated gene dispersal? How do reductions in pollinator visitation rates and S-allele diversity interact in effects on reproductive success (*Young et al., 2012, this issue*)? For plants with sexual polymorphisms, how does pollinator behaviour vary among morphs, and how does this affect the mating system and the relative success of different sexual types (*Ashman, 2000; Case and Ashman, 2009*)? How does sharing pollinators with co-occurring plant species affect the mating system (*Bell et al., 2005; Moeller, 2006*)? Understanding and answering such questions will require thorough studies of pollinator visitation patterns and precise genetic estimation of the mating system, and will provide more comprehensive insights into the evolution of plant reproduction.

Experimental studies of plasticity in selfing rates and sexual polymorphisms

Traits such as herkogamy, floral display size, self-fertility and gender expression often show striking spatial and temporal variation. Yet surprisingly few experimental studies have quantified the effects of environmental variation on the expression of mating-system traits. Additional research on phenotypic plasticity will be particularly helpful for understanding the origin and maintenance of mating-system variation. For example, does plasticity of mating-system traits differ among sites, and is this due to environmental factors such as soil moisture, herbivory and/or pollinator availability (*Ashman 2006; Kameyama and Kudo, 2009; Ivey and Carr, 2012, this issue*)? Does plasticity of mating-system traits vary across a species' range (*Levin, 2012, this issue*)? Do inbreeding species exhibit higher levels of phenotypic plasticity than outcrossing species (*Bradshaw, 1965; Ivey and Carr, 2012, this issue*)? Does the expression of gender plasticity vary with environmental context, and how would such environmental dependence affect the evolution of sexual polymorphisms (*Vaughton and Ramsey, 2012, this issue; Spigler and Ashman, 2012, this issue*)?

Addressing these questions effectively will require innovative experimental designs and strategic choice of study systems. Nonetheless, given that trait variation underpins adaptive evolution, identifying the causes of variation in mating-system traits is crucial for improving our understanding of mating-system evolution.

The future of plant mating system studies in the era of next-generation sequencing

A generation ago research on plant mating systems blossomed with the widespread availability of co-dominant genetic markers (*Brown and Allard, 1970*), facilitating studies of outcrossing rates and patterns of paternity. Today, plant evolutionary biologists are adapting to another revolution in molecular technology, next-generation sequencing (NGS), which again opens up fresh areas of inquiry. For example: does the genetic composition of stigmatic pollen loads differ from the genetic composition of progeny within fruits? Do subtle differences in the floral morphology of pollen donors

lead to spatial heterogeneity in placement of pollen on pollinators? Can we assess frequency-dependent changes in self-incompatibility alleles in natural populations? By taking advantage of current developments in genome sequencing technology we may now be able to address these and other previously intractable questions.

With next-generation sequencing technologies, researchers are increasingly able to genotype hundreds of markers within populations of non-model organisms, and probe the genetic architecture of population and individual traits critical to answering important questions about plant mating systems. The benefits of NGS are already being seen across a wide range of fields including genomics (Hawkins *et al.*, 2010), genetics (Metzker, 2010), human disease (Day-Williams and Zeggini, 2011), and crop genetics and breeding (Varshney *et al.*, 2009). There is much excitement about the potential for NGS to benefit fields closely allied to plant mating systems, including genomic ecology and evolutionary biology (Hudson, 2008), molecular ecology (Eklom and Galindo, 2011) and conservation genetics (Allendorf *et al.*, 2010; Ouborg *et al.*, 2010). What opportunities might NGS offer plant mating system studies?

Rapid cost-effective marker development for mating system and paternity analysis. One far-reaching benefit of NGS that is already being realised across multiple fields is rapid, cost-effective, genome-wide discovery of variable genetic markers such as microsatellites (Dalca and Brudno, 2010; Davey *et al.*, 2011). Gardner *et al.* (2011) offer a helpful procedural guide for ecologists planning to use NGS for marker development. As the technology improves further, the simultaneous genotyping of large panels of microsatellites across multiple individuals will be possible in any species for a fraction of current costs. Thus NGS will enable for the first time cost-effective paternity analysis in large natural populations. This capability could even enable determination of pollen donor representation in the pollen load on a single stigma. Comparison to realized paternity for entire seed families could lead to exciting insights into pollen competition and reproductive incompatibilities.

Contributions of NGS for phylogenetic analysis. The mapping of mating system traits onto a robust phylogeny can reveal important evolutionary insights. For example, Ferrero *et al.* (2012, this issue) discovered phylogenetic evidence for the independent evolution of heterostyly and incompatibility in the Boraginaceae, contradicting the expectation of strong linkage between these traits. Unfortunately, a lack of phylogenetically informative markers often prevents the construction of robust phylogenies, the first step in this process. This is particularly true in recently evolved groups that may offer the most potential for exploring dynamic evolutionary processes (Fig. 8; Hodges and Derieg, 2009; Peakall *et al.*, 2010).

NGS can assist phylogenetic analysis in at least two ways. First, rapid, low-cost phylogenetically informative genetic markers can be found via transcriptome sequencing that targets coding genes (Eklom and Galindo, 2011). Genome-wide phylogenetic analysis can be achieved using restriction-site associated DNA sequencing (RAD-Seq) that offers the potential for detecting 1000s of polymorphisms

simultaneously across multiple individuals in a single NGS run (Davey and Blaxter, 2011; Davey *et al.*, 2011).

Candidate gene discovery. Beyond marker development *per se*, NGS offers exciting new opportunities for fast-tracking candidate gene discovery (Bräutigam and Gowik, 2010). While the genetic bases of self-incompatibility (SI) mechanisms and the loss of SI are increasingly well understood in model systems such as *Arabidopsis* (see Shimizu *et al.*, 2011, for review), NGS may open the door for rapid progress in candidate SI gene discovery in non-model systems. Similarly, we anticipate NGS will facilitate progress on the genetic basis of sex determination in plants, which is central to our understanding of the evolution of separate sexes. Additionally, identifying the loci underlying variation in mating system traits is becoming increasingly accessible outside model systems through developments in NGS. Restriction-site associated DNA sequencing techniques can generate a wealth of data across the entire genome for a sample of individuals in a population. When individual sequences within the population sample are grouped by some measured phenotype, loci linked to the measured trait can be identified (Davey and Blaxter, 2011). Theory for marker-based inferences of the heritability of quantitative traits is already well developed (Ritland, 1996) and, in conjunction with the developing measures of relatedness for population data gleaned from massive batteries of markers (Ritland, 2011; Browning and Browning, 2010), the underlying genetic architecture of floral traits is now within reach.



FIG. 8 The rewardless orchid *Chiloglottis trapeziformis* sexually attracts its specific pollinator, males of the wasp species *Neozeleboria cryptoides*, by a novel semiochemical identical to the sex pheromone of the female wasp. Closely related species of *Chiloglottis* use different chemical variants to attract their own specific male pollinators, suggesting chemical changes may trigger speciation. Next-generation sequencing is now being employed to fast track the discovery of the genes involved in speciation. Photograph by Rod Peakall.

Rapid chloroplast genome sequencing. With next-generation sequencing capability the entire chloroplast genome is becoming an increasingly accessible tool for use in both population and community studies. For example, Doorduyn *et al.* (2011) reported the complete chloroplast genome sequence of 17 individuals within a single pest plant species, *Jacobaea vulgaris*. Although only recently recognized for its potential to reveal genetic variation within species (see Ebert and Peakall, 2009, for review), when combined with predominantly uniparental transmission, genetic variation at the chloroplast is ideally suited for teasing apart pollen and seed-mediated gene flow. Studies that make use of individual levels of cpDNA variation will also shed new light on patterns of both maternity and paternity in dispersed seeds and juvenile plants in natural populations.

Metzker (2010) suggests that the potential applications of NGS are primarily limited by 'one's imagination', and there can be little doubt that NGS offers powerful and exciting new opportunities for advancing the field of plant mating systems. Nonetheless, NGS is not a panacea, nor is it a substitute for employing simple, yet elegant experimental designs, or a justification for abandoning strategically chosen 'model' systems. Indeed a danger of this new revolution is that we lose sight of the important questions in biology (Tautz *et al.*, 2010), perhaps even drowning in the gigabytes of data that will emerge from a single run! Finally, notwithstanding the promise of NGS, there remain many outstanding questions about plant mating systems that can be investigated effectively without the need to conduct genetic or molecular analysis at all, as many studies in this Special Issue demonstrate.

CONCLUSIONS

The remarkable diversity of plant mating systems provides unique opportunities for exploring the dynamics of evolutionary processes. Armed with a strong and growing theoretical basis for predictions, powerful new molecular techniques and creative field experiments, researchers are poised to make many exciting breakthroughs in the coming years. The papers in this Special Issue provide inspiring examples of recent discoveries, and glimpses of exciting developments yet to come.

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