# GENOMIC CONSEQUENCES OF OUTCROSSING AND SELFING IN PLANTS

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Evolutionary transitions from outcrossing to selfing are expected to cause a reduction in the effective population size and a corresponding increase in fixation rates of slightly deleterious mutations and decrease in fixation of advantageous mutations. Despite these predictions, evidence from genomic data does not suggest a significant reduction in the efficacy of selection associated with high levels of self-fertilization. Here, we discuss opportunities for selfing populations to avoid an irreversible decline in fitness toward extinction and the implications for genome evolution. Most directly, large population sizes and the purging of deleterious recessive mutations can reduce genetic loads and slow the effects of genetic drift. Theory suggests that recombination rates may also evolve in response to the evolution of mating system, which can offset the harmful effects of inbreeding. Cytological data supporting the evolution of higher recombination rates in selfing species should be supplemented with genetic and molecular methods for estimating this parameter. Mutation rates may also evolve to be higher in selfing plants as a result of hitchhiking with advantageous mutations, although this is unlikely to lead to increased fitness. Finally, the abundance and activity of selfish genetic elements may also be reduced in selfing lineages, reducing the accumulation of transposable elements, B chromosomes, biased gene conversion, and the spread of cytoplasmic male sterility mutations. This reduction in genomic conflict can increase mean fitness, reduce deleterious mutation rates, and reduce genome size. We show, using comparative data, that highly selfing plants have genomes significantly smaller than those of outcrossing relatives, consistent with reduced activity and spread of repetitive elements in inbred plants. We discuss opportunities for tests of theory as plant genomic data accumulate and argue that a genomic perspective on reproductive transitions in a phylogenetic context should provide important insights into the diversity of reproductive systems in flowering plants.

*Keywords:* inbreeding, genome evolution, recombination, transposable elements, genome size, deleterious mutation.

## Introduction

Flowering plants exhibit a spectacular diversity in reproductive systems, and this can have important effects on the amount and structuring of genetic variation within and among populations (Hamrick and Godt 1996; Glemin et al. 2006). Reproductive transitions, such as the shift in mating system from outcrossing to selfing, tend to increase the extent of linkage disequilibrium, the degree of association among polymorphic sites. When we consider the probability of fixation of mutations subject to natural selection, the strength, efficacy, and sign of selection acting on mutations can be influenced by the extent of linkage disequilibrium with other sites in the genome. Therefore, evolutionary transitions in reproductive systems should play a central role in genome evolution. However, little is known about the genomic consequences of plant reproductive diversity and how transitions in sexual systems and patterns of mating may influence genome evolution.

At last count, there are 41 large-scale plant genome sequencing projects under way (http://www.ncbi.nlm.nih.gov/genomes/leuks.cgi), with more to follow in the next few years. Although many of these projects focus on crop plants, increasing atten-

tion is being focused on ecological and evolutionary model systems, including *Mimulus guttatus*, *Aquilegia formosa*, *Thellungiella halophila*, *Capsella rubella*, and *Arabidopsis lyrata* (http://www.jgi.doe.gov/sequencing/allinoneseqplans.php). As our ability to compare patterns of genome structure and evolution accelerates, a theoretical and empirical framework for understanding plant genome evolution in wild plant populations becomes increasingly important. Moreover, because many of these nondomesticated species display extensive variation in reproductive traits and patterns of mating, there are likely to be rich opportunities for investigating the genomic consequences of reproductive diversity in flowering plants.

Although extensive research has focused on genome evolution in polyploids (Chen 2007), relatively little attention has focused on the potential for mating system transitions to restructure genomes, perhaps because the effects of polyploidy are immediate and more conducive to experimental manipulation (Husband et al. 2008). In contrast, mating system transitions are expected to lead to shifts in the selective dynamics of genomic elements over evolutionary timescales. Nevertheless, such changes could be equally important for understanding the evolutionary dynamics of plant genomes.

Mating system variation has several important effects on the genetic properties of populations (fig. 1), which we treat here only briefly because they have been covered in several recent
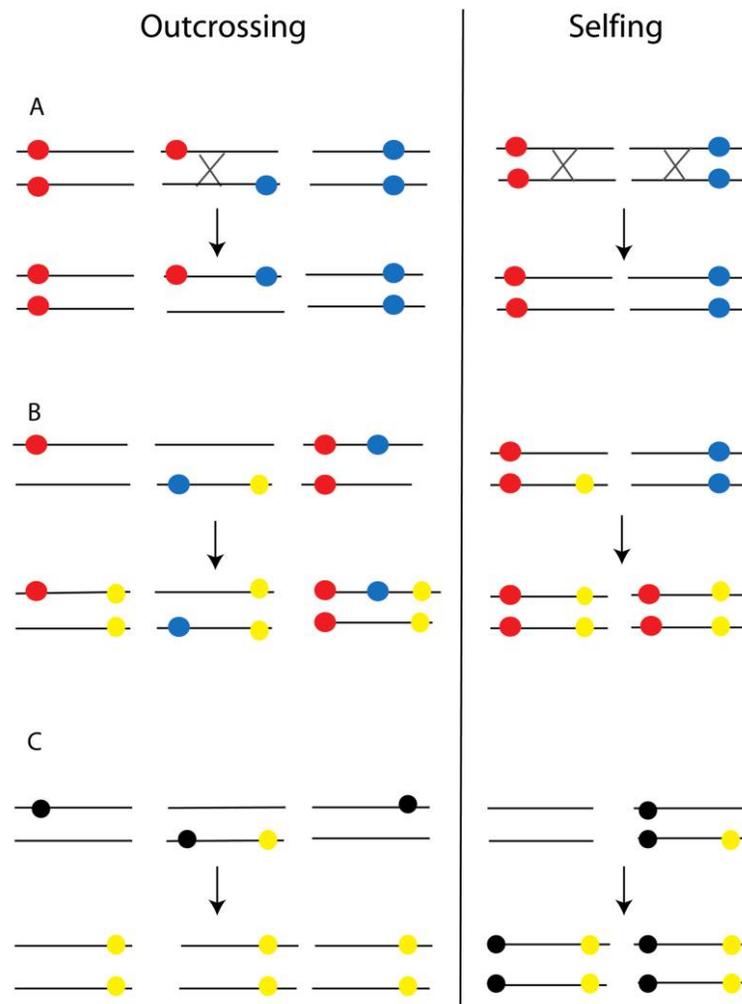
**Fig. 1** Expected effects of outcrossing and selfing on patterns of genetic variation and molecular evolution. *A*, Effects on linkage disequilibrium. The blue and red circles represent neutral polymorphic mutations, and each pair of lines represents a diploid individual. In outcrossing species, polymorphic sites will often be found in heterozygotes, and crossing over between polymorphic sites can generate novel haplotypes, breaking up linkage disequilibrium. With selfing, although physical crossing over occurs, the low level of heterozygosity leads to an effective reduction in recombination rate, maintaining linkage disequilibrium. *B*, Effects on diversity. The yellow circle represents an advantageous mutation arising in one chromosome. In outcrossers, the effectively high rate of recombination can uncouple the fate of the advantageous mutation from linked variation, maintaining neutral variation as the advantageous mutation gets fixed. With selfing, the fixation of the advantageous mutation is accompanied by fixation of linked neutral variants, due to hitchhiking. *C*, Effects on the fixation of deleterious mutations. Deleterious mutations are shown in black. With outcrossing, the deleterious mutations can be eliminated by selection independently of the fixation of an advantageous mutation. With selfing, fixation of the advantageous mutation can be accompanied by fixation of a linked deleterious mutation, provided that the net selection coefficient is highest on the linkage group with both the advantageous and the deleterious mutations.

reviews (Charlesworth and Wright 2001; Glemin et al. 2006). Most directly, homozygosity increases as a function of selfing rate, and this reduces the effective size of a population ($N_e$) as a result of a reduction in the number of distinct alleles. The effective size of a completely selfing population is reduced twofold as a result of homozygosity (Charlesworth et al. 1993; Nordborg 2000). Further, because of homozygosity, crossing over rarely occurs between heterozygous sites, increasing linkage disequilibrium among loci (figs. 1*A*, 2; see Nordborg 2000). This results in stronger effects of genetic hitchhiking, in the form of selective sweeps of positively selected mutations (fig. 1*B*) and back-

ground selection acting against deleterious mutations (reviewed in Charlesworth and Wright 2001), which further reduce $N_e$ in the affected regions. Linkage among weakly selected sites with opposing selective forces can also interfere with the ability of selection to act efficiently (McVean and Charlesworth 2000). All of these forces reduce $N_e$ and may be further exaggerated by life-history characteristics associated with selfing that promote population subdivision, isolation, and genetic bottlenecks. Finally, highly selfing species may experience reduced levels of between-species introgression (Sweigart and Willis 2003), leading to further reductions in genetic diversity.
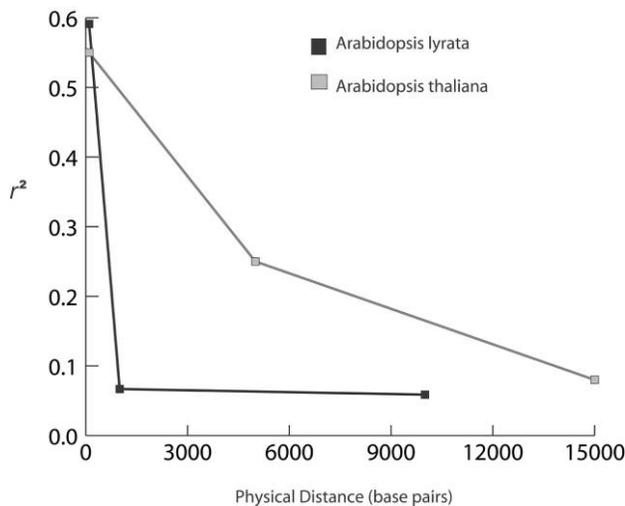
**Fig. 2** Decay of linkage disequilibrium with physical distance in the highly selfing *Arabidopsis thaliana* and the self-incompatible *Arabidopsis lyrata*. The *Y*-axis depicts the average squared correlation coefficient between pairs of polymorphic sites, and the *X*-axis represents their physical distance. These data, as well as data from species-wide samples, are reported by Wright et al. (2006) and Nordborg et al. (2005) for *A. lyrata* and *A. thaliana*, respectively.

Together, these processes should lead to a decrease in the efficacy of natural selection and an increase in the fixation rate of slightly deleterious mutations (fig. 1C), with important consequences for evolution at the genome level (Lynch and Conery 2003). Over evolutionary time, increased deleterious mutation accumulation can be important in causing species extinction (Lynch et al. 1995), and this may, in part, explain the lack of persistence of selfing lineages, as revealed from comparative and phylogenetic studies (reviewed in Takebayashi and Morrell 2001; Igic et al. 2008).

There is clear and consistent evidence for a reduction in levels of within-population neutral diversity in highly selfing species (Schoen and Brown 1991; Glemin et al. 2006). In contrast, to date, studies of molecular evolution have found only limited evidence for elevated fixation of deleterious mutations in selfing species (Wright et al. 2002; Glemin et al. 2006). One possible explanation for this is the inadequacy of sampling to date. There is simply a need to collect data from very large numbers of consistently sampled loci, given the inherent stochasticity of deleterious mutation accumulation. This will soon become feasible with the rapid expansion of whole-genome data. Alternatively, the timescale for the evolution of selfing may be too recent in many lineages for substantial genomic changes to have occurred. However, it is also possible that many selfing lineages, particularly those successful model systems that have been the focus of research, can avoid long-term fitness decline through compensatory mechanisms.

In this review, we examine the potential for avoidance of fitness decline associated with the evolution of self-fertilization. First, elevated homozygosity, particularly in large populations, can lead to the purging of recessive, strongly deleterious mutations and can enhance the fixation of recessive advantageous mutations. Second, recombination rates are predicted to evolve after the evolution of selfing, which can directly reduce the harmful effects of suppressed recombination. Third, mutation rates may evolve to be higher; although this could increase rates of adaptation, it may also contribute to mutational meltdown and thus is unlikely to lead to increased fitness in selfing lineages. Finally, selfish genetic elements, which represent a major class of deleterious genomic mutation, may be more effectively selected against in highly selfing populations. Theory predicts that the spread and effects of transposable elements (TEs), B chromosomes, biased gene conversion (BGC), and cytoplasmic male sterility (CMS) mutations should all be reduced in highly selfing species. Because these elements can spread in outcrossing species despite fitness costs, their elimination in selfing lineages can increase mean fitness and reduce genome size and deleterious mutation rates. We now consider these issues in turn.

## Inbreeding and the Efficacy of Natural Selection

Reductions in effective population size in inbreeders due to the factors discussed previously are expected to elevate fixation rates of deleterious mutations and decrease fixation rates of advantageous mutations. However, the degree to which polymorphism and/or substitution rates are affected by selfing will depend strongly on the distribution of mutational effects on fitness, which remains very poorly understood (Wang et al. 1999; Weinreich and Rand 2000; Eyre-Walker and Keightley 2007). In particular, high homozygosity due to inbreeding can lead to greater expression of deleterious recessive mutations, leading to their elimination, a phenomenon referred to as purging (Crnokrak and Barrett 2002; see Schoen and Busch 2008). Because of this, selfing leads to a rapid elimination of highly recessive, strongly deleterious mutations from the population. Models show that deleterious mutation accumulation in inbreeding taxa is dominated by slightly deleterious mutations that are closer to additive in their fitness effects (Wang et al. 1999; S. Glemin, unpublished manuscript). If a large fraction of deleterious mutations are recessive and strongly selected, the effects of purging may thus dominate over slightly deleterious mutation accumulation, at least in the context of studies of molecular evolution. Furthermore, recessive advantageous mutations can be fixed more effectively in highly selfing populations (Charlesworth 1992; S. Glemin, unpublished manuscript), increasing rates of adaptive evolution. In general, the predictions for molecular evolution rely on the assumption of the presence of a large class of mutations that are weakly selected and that are nearly additive in their fitness effects, mitigating any influence of homozygosity in purging deleterious recessive mutations and enhancing the fixation of recessive advantageous mutations (S. Glemin, unpublished manuscript).

In addition to the parameters associated with deleterious and advantageous mutations, population sizes can also be important in predicting the rate of fitness decline. Because the extent of linkage disequilibrium is determined by $4N_e r(1 - s)$, where $r$ is the rate of recombination and $s$ is the selfing rate (Nordborg 2000), high selfing rates can be partially compensated for by large population sizes, and this will reduce any expected effects on diversity and molecular evolution. Even in highly selfing populations with large population sizes, linkage

disequilibrium may not extend over large genomic regions, and the efficacy of natural selection may not be low.

Reduced effectiveness of selection on amino-acid-altering mutations can be inferred by increases in the proportion of nonsynonymous relative to synonymous substitutions (Wright et al. 2002) or polymorphisms (Bustamante et al. 2002). In addition, a reduced efficacy of selection on biased codon usage can be detected via elevation of unpreferred over preferred synonymous substitutions and polymorphisms (Marais et al. 2004). A comparison of substitution rates and codon bias between the highly selfing *Arabidopsis thaliana* and the self-incompatible *Arabidopsis lyrata* failed to find evidence for an effect of mating system for both amino acid substitutions and patterns of codon bias (Wright et al. 2002). This suggests that there is not a major decline in effectiveness of selection, at least in these taxa. Similarly, in a comparative analysis of nuclear and chloroplast DNA sequence data from multiple species, Glemin et al. (2006) found no effect of inbreeding on codon usage, with the exception of comparisons in the Poaceae. In this family, differences in base composition were observed for both synonymous sites and noncoding sites, suggesting that this result is unlikely to be caused by differences in selection on codon usage bias.

Although the above comparisons indicate little effect of inbreeding on the effectiveness of selection, these tests focused on effects on substitution rates between species. Such tests would not detect elevation of slightly deleterious mutations if selfing evolved recently, nor could they reveal any reduction in positive selection on amino acids in selfers because they assume that nonsynonymous substitutions are predominantly slightly deleterious in their effects. A high proportion of rare nonsynonymous polymorphisms has been reported in the inbreeding wild barley *Hordeum vulgare* ssp. *spontaneum* (Cummings and Clegg 1998) and *A. thaliana* (Bustamante et al. 2002; Nordborg et al. 2005), although explicit comparisons of polymorphism and divergence with related outcrossing species have not, as yet, been undertaken. Using a Bayesian analysis of the ratios of polymorphism to divergence for amino acid replacement and synonymous changes, Bustamante et al. (2002) found evidence for excess amino acid polymorphism and a reduction in positive selection in selfers. However, that study compared *A. thaliana* with *Drosophila melanogaster*, and clearly, more detailed analyses of polymorphism and divergence for close relatives with contrasting mating systems are needed.

In their analyses of sequence variation in plant species with contrasting mating systems, Glemin et al. (2006) detected a weak but significant elevation in the ratio of amino acid to synonymous polymorphism in species with high selfing rates. This suggests that there may be an elevated frequency of mildly deleterious polymorphisms segregating in selfing species. However, much of the data they reviewed are restricted to a small number of taxa and/or loci, and direct comparisons of polymorphism and divergence for the same loci are sparse. Large-scale sampling of polymorphism and divergence among close relatives will be important in further assessing whether inbreeding plays a significant role in coding sequence evolution. Currently, comparisons of patterns of amino acid polymorphism and divergence in *Arabidopsis* do not suggest a significant difference between selfing and outcrossing species (J. P. Foxe, H. Zheng, M. Nordborg, B. S. Gaut, and S. I. Wright, unpublished data).

Limited evidence suggests that there may be an elevation of nonsynonymous polymorphism with increased selfing. However, there is little to indicate that inbreeding leads to a sufficient decline in the effectiveness of selection to influence substitution rates and genome evolution. This suggests that there may be relatively few sites experiencing sufficiently weak additive selection to be affected by changes in selfing rate. As a result, the power to detect subtle changes in the patterns of molecular evolution may be too low, given the sample of genes studied to date.

In addition, large population sizes may reduce linkage disequilibrium in the selfing taxa studied. *Arabidopsis thaliana* is a predominantly selfing annual with a near-worldwide distribution. This successful colonizing species exhibits high nucleotide diversity and retains a fairly rapid decay of linkage disequilibrium with physical distance in rangewide population samples, despite very high selfing rates (Nordborg et al. 2005). Although within-population diversity is much lower in *A. thaliana* than in populations of the self-incompatible *A. lyrata* (Savolainen et al. 2000; Wright et al. 2003b; Ramos-Onsins et al. 2004), this does not necessarily imply a general reduction in the efficacy of selection. If selection against slightly deleterious mutations is effective in a metapopulation-type structure, the effective size across populations in *A. thaliana* could counteract reduced effective recombination rates. Furthermore, outcrossing populations can also experience population bottlenecks, reducing variation and increasing linkage disequilibrium to levels comparable to those of inbreeders (Wright et al. 2003b). A very broad taxonomic comparison is illustrative; humans have an order of magnitude lower level of variability and much more extensive linkage disequilibrium than do worldwide samples of *A. thaliana* (Nordborg et al. 2005), and they show a comparable, or more severe, genome-wide excess of slightly deleterious amino acid polymorphism (Bustamante et al. 2005). Similarly, low linkage disequilibrium has recently been demonstrated in highly selfing wild barley populations (Morrell et al. 2005). In contrast, recent comparisons of outcrossing and selfing species of *Caenorhabditis* identified very extensive levels of linkage disequilibrium in the selfing *C. elegans*, compared with outcrossing congeners (Cutter et al. 2006). Thus, variation in the population history of a species may contribute to variation in fixation rates at weakly selected sites as much as, or more than, mating system, swamping out any effect of the latter.

The role of purging and large population sizes in eliminating deleterious mutations in selfing populations may explain the contrast between the results to date for selfing species and those found for nonrecombining Y chromosomes in plants and animals, where recurrent evidence for deleterious mutation accumulation has been found (Charlesworth and Charlesworth 2000). In inbreeders, deleterious mutations are fully expressed as homozygotes, so fixation is directly dependent on the selection coefficient. In contrast, because of the persistence of functional gene copies on the X chromosome, Y chromosome fixation probabilities will be determined by $hS$, the product of the selection coefficient ($S$) and the dominance coefficient ($h$). In the extreme case, mutations that are completely recessive in their fitness effects can fix neutrally on Y chromosomes, despite strong selection coefficients in homozygotes. Thus, if deleterious mutations are strongly recessive, the permanent heterozygosity of the Y chromosome brings a much larger proportion of

deleterious mutations into a parameter space that allows their fixation. Perhaps even more important, the non-recombining portion of Y chromosomes exhibits near-complete recombination suppression, whereas highly selfing species can more easily escape the linkage effects, for example, by having larger population sizes. The complete suppression of recombination in Y chromosomes leads to much stronger hitchhiking than is experienced even with very high selfing in large populations.

## Evolution of Recombination Rate

The increase in homozygosity as a result of selfing reduces opportunities for recombination to break down associations among alleles by crossing over between heterozygous loci (Nordborg 2000). This increases linkage disequilibrium and decreases the effective rate of recombination. However, few species that engage in high levels of selfing are exclusively selfing (Barrett and Eckert 1990; Igic and Kohn 2006), and therefore, some opportunity exists for recombination during rare outcrossing events. It is therefore possible that natural selection may favor modifiers that increase rates of physical recombination or crossing over in selfers to offset the effects of inbreeding. Although theory predicts that mating systems may play an important role in the evolution of recombination rates, this area has received relatively little empirical attention.

Recombination is generally thought to be advantageous because it breaks down associations between alleles (linkage disequilibrium) and is favored under several non–mutually exclusive conditions, for example, with weak negative epistatic associations among mutations (Feldman et al. 1980; Kondrashov 1982, 1988; Barton 1995), when directional selection negatively covaries between habitats (Lenormand and Otto 2000), and when interference among selected sites is strong, such as in populations with small effective population size (Fisher 1930; Muller 1932; Otto and Barton 1997, 2001; Barton and Otto 2005; Keightley and Otto 2006). Under these conditions, recombination increases the variance in fitness and can introduce higher-fitness alleles on the same genetic background.

Models exploring the fate of recombination modifiers have mostly assumed random mating, and little attention has been given to how the mating system may alter the outcome of these models. Simulations indicate that hitchhiking between a recombination modifier and a pair of selectively important loci is stronger with selfing and that this difference can, under some conditions, favor the evolution of higher recombination rates. In contrast, the recombination rate is generally driven downward with random mating (Charlesworth et al. 1977, 1979; Holsinger and Feldman 1983a). Supporting these results, Roze and Lenormand (2005) generated an analytical model and found that even small amounts of selfing can greatly increase the range of parameters under which selection favors increased recombination.

In small populations, genetic drift is primarily responsible for generating negative linkage disequilibrium that favors recombination modifiers, increasing rates of crossing over (Otto and Barton 2001). The strength of indirect selection is stronger when linkage among loci is tight and population size is small; with tight linkage, a broader parameter space of population size favors recombination modifiers (Barton and Otto 2005). Although not modeled explicitly, these results suggest that with

self-fertilization, there may be stronger selection on recombination modifiers. Although details of the models vary, there is a general agreement that a fairly broad set of conditions favor an increase in the recombination rate with increased selfing. Support for these predictions is provided by comparisons of chiasma frequency in plant species with contrasting mating systems (Ross-Ibarra 2004; Roze and Lenormand 2005).

An alternative method for estimating the rate of crossing over per base pair is to integrate genetic linkage maps with physical maps. This allows for estimates of the average frequency of crossing over per physical length and a more detailed view of rate heterogeneity in different genomic regions, although the accuracy of cytological versus genetic estimates of recombination rate has been subject to debate (Nilsson et al. 1993). Such integrated maps are available for several model systems and agricultural taxa. However, the only detailed comparison of map-based recombination rates in closely related species with contrasting mating systems is between *Arabidopsis thaliana* and *Arabidopsis lyrata* (Kuittinen et al. 2004; Hansson et al. 2006; Kawabe et al. 2006). As predicted, overall rates of recombination per unit physical length are higher in *A. thaliana*. The degree of difference in recombination rate between the two *Arabidopsis* species varies across different genomic regions.

In the *Arabidopsis* comparison, the contrast in overall rate of recombination is complicated by a shift in genome size. *Arabidopsis thaliana* has a reduced genome size, and there is a general trend that rates of recombination per base pair decrease with increasing genome size (Ross-Ibarra 2006). Therefore, it is difficult to rule out an effect of genome size difference rather than direct selection on recombination modifiers. *Arabidopsis lyrata* has a larger genome and potentially more heterochromatic, nonrecombining DNA than *A. thaliana*. Therefore, the difference in average recombination rates across large regions could result from other aspects of genome evolution in addition to selection on recombination modifiers, such as differences in the accumulation of TEs. Nevertheless, using phylogenetically independent contrasts of 142 species with genome size as a covariate, Ross-Ibarra (2006) detected elevated chiasma frequencies in selfers, suggesting higher rates of recombination than in related outcrossers. In addition to overall recombination frequencies, outcrossing rates have also been shown to correlate with the ratio of recombination rates in female function relative to male function. Lenormand and Dutheil (2005) reported that selfing species tend to have a higher male : female ratio of recombination rates than outcrossing species. Their interpretation is that because pollen competition is reduced in selfing species, this relaxes selection against recombination breaking up favorable epistatic allelic combinations in pollen. Given that most estimates of chiasma frequencies have been determined in pollen, it will be important to test whether sex-averaged recombination rates are generally elevated in selfing species.

## Relations between Mating System and Genomic Mutation Rate

Beneficial mutations are ultimately necessary for novel adaptations to evolve. However, the relative input of beneficial and deleterious mutations determines the evolution of genome-wide mutation rate, which may be strongly influenced by mating

system. In a random-mating population, a modifier that increases the mutation rate should be selected against because it introduces more deleterious than beneficial mutations. Therefore, the fact that estimates of mutation rate are all above zero presumably reflects physical constraints on further reductions or a trade-off between fitness effects of new deleterious mutations and the amount of energy required for higher-fidelity DNA replication and repair (see Sniegowski et al. 2000). However, in selfing and asexual populations, strong linkage between a modifier that increases the mutation rate and a resulting beneficial mutation may result in indirect selection for the modifier. It is therefore possible to achieve an equilibrium rate of mutation that is above zero without invoking any physical constraints. The equilibrium value depends both on the extent of linkage disequilibrium and on the relative frequency and effect of deleterious and beneficial mutations. However, while many models have investigated the effects of indirect selection in asexual populations (Kimura 1967; Leigh 1970; Eshel 1973; Painter 1975; Woodcock and Higgs 1996; Orr 2000), only one has explicitly modeled the effect of self-fertilization (Holsinger and Feldman 1983*b*). Furthermore, there is a paucity of empirical tests of these predictions. We summarize advances made in understanding the effect of mating system, specifically selfing, on the evolution of genomic mutation rate.

The effective rate of recombination in highly selfing populations is low. Therefore, a modifier that increases the mutation rate will have a higher probability of linkage to a beneficial mutation and will be indirectly selected. In a highly selfing population, a modifier locus that alters the mutation rate at a second locus is predicted to reach a nonzero equilibrium mutation rate when overdominance favors the novel heterozygote genotype (Holsinger and Feldman 1983*b*). This result is robust for a nontrivial amount of outcrossing (<10%), making it more relevant to natural systems, which, as discussed earlier, are rarely, if ever, completely selfing. Johnson (1999) considered a mutation modifier that generates mutations of varying effect across a genetic map instead of one that acts on a single selectively important locus. With smaller map sizes, beneficial mutations will have a stronger influence on the fate of a modifier than previously predicted. Interestingly, the strength of linkage disequilibrium, which is of primary importance, is much higher for selfers than for random-mating populations. This suggests that if a high proportion of mutations are beneficial, they may be more important in predominantly or partially selfing populations. However, if beneficial mutations are very rare and weakly selected, then deleterious mutations will cause stronger negative selection against increased mutation rates in selfing (or asexual) populations than in outcrossing populations (Kondrashov 1995; McVean and Hurst 1997; Dawson 1998). To distinguish between these possibilities, the distribution of mutational effects is required, yet empirical estimates of the relative proportion of beneficial and deleterious mutations are largely unavailable.

Rates of deleterious mutations between predominately outcrossing *Amsinckia douglasiana* and selfing *Amsinckia gloriosa* have been compared in a mutation accumulation (MA) experiment (Schoen 2005). Although both lineages showed declines in fitness because of an accumulation of mutations, there was no significant difference associated with the mating system. Only three other MA experiments have been conducted in plants,

two with *Arabidopsis thaliana* (Schultz et al. 1999; Shaw et al. 2000) and another with *Triticum durum* (hard wheat; Bataillon et al. 2000). A problem inherent to MA studies is the difficulty of conducting experiments with organisms with long generation times. Because most mutations are of small effect, many generations are required for the power necessary to detect differences among groups. MA studies are primarily concerned with the rate of deleterious mutations and therefore underestimate the total mutation rate across the genome. Further, the exact measure of $U$ (deleterious mutations per genome per generation) is dependent on the underlying distribution of mutational effects, and estimates of $U$ can vary by orders of magnitude, depending on the distribution that is assumed (Shaw et al. 2000, 2002).

An alternative method of estimating mutation rates is by comparing nucleotide sequences of divergent lineages. The level of neutral divergence between two lineages is equal to $2\mu t$, where $\mu$ is the mutation rate (per nucleotide per generation) and $t$ is the number of generations since divergence (Kimura 1968). However, this assumes a constant mutation rate and is therefore not useful for testing whether there are rate differences between the lineages. The relative rates test (Sarich and Wilson 1967; Wu and Li 1985; Tajima 1993) can be used to test for heterogeneity of rates among lineages. While there are a number of studies that apply this technique to noncoding and silent sites of nuclear loci in plants (e.g., Gaut et al. 1996, 1999; Wright et al. 2002; Senchina et al. 2003), there are none that do so for enough loci to estimate a mean mutation rate across the genome. In fact, substantial heterogeneity of mutation rates among loci in the above studies demonstrates the necessity of using many loci to estimate a genomic mutation parameter. A parsimony-based study of substitution rates at 23 genes failed to detect a significant difference in neutral mutation rate between *A. thaliana* and *Arabidopsis lyrata* (Wright et al. 2002). A follow-up comparison did suggest a slight but significant elevation of synonymous substitutions in *A. thaliana* using a sample of 83 genes (Wright 2003). This contrast is complicated by differences in generation time because *A. thaliana* is annual and *A. lyrata* is biennial or perennial. However, Whittle and Johnston (2003) found no association of generation time with mutation rate in a comparison of 24 phylogenetically independent pairs of annual and perennial plants. Complete uncoupling of generation time from mating system will require broader-scale comparisons in a phylogenetic context.

Could higher mutation rates enhance the ability of selfers to adapt, counteracting the harmful effects of inbreeding? Both theory and data suggest that this is unlikely. In particular, because most mutations are deleterious, elevated mutation rates are likely to be transient outcomes of hitchhiking with beneficial mutations rather than long-term shifts that increase adaptation (Sniegowski et al. 2000). Overall, higher mutation rates should enhance the mutational meltdown predicted in inbreeding populations rather than reduce its deleterious consequences.

## Mating Systems and Genetic Conflicts

"Selfish" genetic elements that enhance their own transmission, despite null or negative fitness consequences for the genome, represent a dominant component driving evolutionary divergence in eukaryotic genomes (Burt and Trivers 2006).

The persistence and spread of selfish genetic elements can be generally understood as a balance between their transmission advantage and any deleterious effects related to their activity. If the rate of spread of selfish genetic elements predominates over reduced fitness, such elements can persist and even become fixed in natural populations. Their activity can also represent a significant contribution to spontaneous mutation rates and genetic load (Lai et al. 1994; Houle and Nuzhdin 2004).

As we will discuss, transitions in mating system, including increased rates of selfing, are generally expected to have an important effect on the outcome of genomic conflicts. In general, highly inbred or asexual taxa are expected to experience reduced intragenomic conflict because the lack of outcrossing prevents the spread of selfish genetic elements into other genetic backgrounds, thereby increasing the variance in fitness and leading to stronger purifying selection (Cavalier-Smith 1980; Hickey 1982). Furthermore, because selfish genetic elements will remain in greater linkage disequilibrium with replicate elements causing harmful mutations, there may be selection on the elements themselves for reduced activity, as models have shown for the evolution of transposition rates (Charlesworth and Langley 1996). Finally, because many selfish genetic elements increase transmission rates by gaining overrepresentation in gametes when competing with alternative alleles, the presence of high homozygosity in selfing populations acts as a further deterrent to alleles that make use of meiotic drive to increase in frequency. With homozygosity, the competition among alleles that leads to drive is absent because individuals have two copies of either the driving allele or the wild-type allele but not both. Therefore, selfing ensures a second level of homogeneity not experienced by asexuals and should act further to inhibit the spread of selfish elements. Given the potential importance of selfish genetic element activity for mean population fitness and deleterious mutation rates, selective elimination of selfish elements by inbreeders could offset the predicted decline in fitness associated with selfing.

### Transposable Elements

Equilibrium models of TE evolution indicate that stable copy numbers can be maintained in populations by a balance between transposition increasing copy number and the action of negative selection removing insertions from the population (Charlesworth and Langley 1989). In inbreeders, computer simulations (Wright and Schoen 1999) and analytical models (Morgan 2001) show that the spread and accumulation of TEs can be inhibited by the lack of outcrossing as a result of the reduced spread of elements between individuals, as well as the purging of insertions with deleterious recessive effects on fitness. As mentioned previously, self-regulated transposition is also more likely to evolve in inbreeders (Charlesworth and Langley 1996), bringing down the copy number compared with that of outcrossing relatives.

By contrast, if natural selection acts predominantly against TE insertions through ectopic (i.e., between-element) recombination events, causing chromosomal rearrangements in heterozygotes, there may be a strong relaxation of natural selection against TEs in selfers (Charlesworth and Charlesworth 1995). This could lead to rapid accumulation and population fixation (Wright and Schoen 1999; Morgan 2001). The net outcome will depend on the underlying nature of selection on TEs and the history of selfing. For example, recent transitions to inbreeding may relax selection against ectopic exchange and may reduce effective population size, leading to increased frequencies and fixation of insertions. However, over the long term, the inability of new insertions with deleterious effects to spread through selfing populations may limit a rampant accumulation process. Simulations show that even under the ectopic exchange model, stochastic loss of elements from selfing populations is more frequent, potentially resulting in a net loss of TEs from selfing genomes (Wright and Schoen 1999). With a recent transition to selfing, this could lead to an effect in one direction on polymorphism patterns (i.e., increased TE frequencies at individual sites inherited from the ancestor), without a large copy number increase or with, perhaps, a decrease in copy number in selfers. Note that in either case, TE activity reduces fitness less in inbreeding than in outbreeding populations.

Early results suggest a decline in TE abundance in selfing species, consistent with the basic predictions of deleterious insertion models. Morgan (2001) reviewed evidence from a number of species pairs, showing a general reduction in copy number in inbreeding species. Furthermore, given the strong correlation between TE abundance and genome size, preliminary evidence for reduced genome size in selfers is at least consistent with inbreeding playing a role in the elimination of selfish genetic elements. In a population genetic study, Ac-like TE insertions were at higher frequencies in populations of the selfing *Arabidopsis thaliana* compared with *Arabidopsis lyrata* (Wright et al. 2001). This suggests a relaxation of natural selection, but this was not accompanied by an accumulation of new element insertions, consistent with inhibition of new element activity. Similar results were obtained in a study of retrotransposable elements in tomato (Tam et al. 2007), although a lack of evidence for purifying selection for most transposons, even in outcrossing taxa, suggests that the patterns may reflect primarily neutral insertion polymorphism. Analysis of TE distributions in *A. thaliana* also suggested that recombination rate heterogeneity does not appear to influence TE accumulation in this species, as has been observed in outcrossing genomes (Wright et al. 2003a), and this pattern is also apparent in the selfing nematode *C. elegans* (Duret et al. 2000). Selection against ectopic recombination may be a weak force in most selfing genomes.

As with inbreeding, asexual species are expected to show reduced TE activity and abundance, particularly as a result of the inability of active elements to spread among individuals (Hickey 1982). In contrast to this prediction, analysis of sequence evolution of three retrotransposon families in four asexual plant species identified many copies and evidence for selective constraint on TEs in these taxa, suggesting that elements remain active (Docking et al. 2006). However, given the likely recent evolution of asexuality in these plant species (Docking et al. 2006), simulations indicated that the evidence for residual selective constraint in TEs was not unexpected, even if long-term reductions in activity are occurring. More direct comparisons of abundance and polymorphism patterns between related sexual and obligate asexual taxa would be useful to provide a direct test of the effects of transitions to asexuality on TE evolution.

## B Chromosomes

B chromosomes are nonessential chromosomes found in addition to the basic set of chromosomes. They have now been identified in more than 2000 species (Burt and Trivers 2006), including more than a thousand plant species (Jones 1995), are often morphologically distinct, are usually smaller than essential chromosomes, and show numerical variation within and between individuals. B chromosomes may be neutral, positive, or, more often, harmful in their effects and are maintained in the genome via a meiotic drive mechanism, ensuring a greater representation in gametes than expected by chance (Camacho 2006).

Models have shown a strong effect of outcrossing rate on the equilibrium frequencies of B chromosomes as a result of reduced transmission and greater variance in fitness. For example, Burt and Trivers (1998) found that outcrossing rates below 50% lead to complete elimination of B chromosomes from populations, reducing significant fractions of "junk DNA" and any fitness costs associated with B chromosome maintenance. A comparative survey of the distribution of B chromosomes in 353 plant species from the United Kingdom (12.5% of which contained B chromosomes), using phylogenetically independent contrasts, confirmed a strong and consistent effect of mating system on the presence of B chromosomes (Burt and Trivers 1998). Three independent analyses performed to test for an association between the presence of B chromosomes and mating system revealed a positive correlation between outcrossing and the presence of B chromosomes in 16 out of 19 taxonomic contrasts. Three predominantly inbreeding species, namely, *Desmazaria rigidum*, *Poa annua*, and *Luzula campestris*, were found to contain B chromosomes. In these cases, it is possible that B chromosomes are beneficial. Alternatively, the timescale of mating system evolution may have been too recent for selection to have effectively purged these genetic elements. Theoretical work incorporating the transition to selfing will be important to better understand the timescales required for the selective elimination of B chromosomes after a shift in mating system.

## Biased Gene Conversion

Gene conversion is the nonreciprocal copying of one stretch of DNA into another during recombination (Marais 2003). It has been argued that the genetic systems involved in this repair are biased and can lead to transmission distortion in favor of GC bases. For example, if an individual is heterozygous at a site for a G/T polymorphism, BGC will lead to an overrepresentation of G over T gametes, thus leading to biased transmission. The net effect is a selective advantage of GC over AT bases, with the selection coefficient being determined by the rate of BGC (Marais 2003). Several studies to date, using patterns of genome structure, population genetic data, and evidence from DNA mismatch repair processes, have found evidence for BGC in yeast (Birdsell 2002), *Drosophila* (Galtier et al. 2006), and mammals (Duret et al. 2006). However, to date, there is little evidence for the presence of BGC in plant genomes.

Because of the transmission advantage inherent in the process, GC bases under this model are effectively selfish genetic elements, where there is a fixation bias toward GC bases as a result of biased transmission. Because the process leads to an increased fixation probability of GC bases, it can have important consequences for base composition evolution. GC-BGC could lead to reduced fitness at sites under weak selection favoring A-T nucleotides, such as plant introns (Ko et al. 1998).

In an outcrossing species, the efficacy of BGC is given by the product $N_e\gamma c$, where $\gamma$ is the probability per generation that a given site is affected by a gene conversion tract $L$ and $c$ is the bias in favor of the GC allele (Nagylaki 1983a, 1983b; Galtier et al. 2001). However, because BGC will occur only in heterozygotes, the effective rate of this process will be reduced dramatically in highly selfing species. Analytical results have shown that the strength of BGC, and thus GC content, will be reduced as a direct product of the selfing rate; because BGC will occur only in heterozygotes, an organism with an outcrossing rate of 1% will experience only 1% the level of BGC of an equivalent highly outcrossing species (Marais et al. 2004). As a result, the mating system could play a role in eliminating any effect of this process on base composition.

If BGC plays a major role in structuring genomic base composition, inbreeding species should generally evolve to become more AT rich, particularly at sites less constrained by other forms of selection, e.g., synonymous sites. In their analyses of genomic diversity in angiosperms, Glemin et al. (2006) examined the GC content of 10 species with contrasting mating systems. Although no effects were identified in most comparisons, outcrossing species in the Poaceae were found to have GC content significantly higher than that of selfers, as measured by total GC, GC at third-codon positions, and GC in introns. Given the consistent elevation of GC across noncoding and coding sites, this pattern is unlikely to be explained by a difference between species in the efficacy of natural selection on codon usage bias. Instead, it is more likely to be the result of contrasting rates of BGC.

Similarly, an analysis of base composition in the Brassicaceae revealed a consistent reduction in GC content at third-codon positions in *A. thaliana* compared with outcrossing *Brassica oleracea* and *A. lyrata* (Wright et al. 2007). Analysis of base composition evolution controlling for gene expression and codon preferences indicated that the contrasting patterns were unlikely to result from differences in the effectiveness of selection on codon usage. Instead, the pattern appeared to be the direct result of a consistent decline in GC richness in *A. thaliana*, potentially as a result of a reduction in BGC following the recent evolution of selfing in this lineage (Nasrallah et al. 2004). Although alternative explanations, including shifts in the patterns of mutation bias, are possible, the difference is consistent with expectations under BGC. Comparisons of polymorphism and divergence for GC⟶AT versus AT⟶GC changes should help untangle the role of BGC versus mutation bias. In particular, if BGC is strong, AT⟶GC changes are expected to segregate at higher frequencies than GC⟶AT changes. This should result in higher frequencies and fixation rates of AT⟶GC changes in outcrossing species compared with selfing species, under the model of BGC. By contrast, no such effect is expected if the difference is driven by changes in mutation bias.

In addition to an overall reduction in GC content, the reduced opportunity for BGC could act to reduce heterogeneity in base composition across the genome in selfers. In particular, variation in GC content may be driven, in part, by variation

in rates of recombination, assuming a tight correlation between estimated rates of recombination and rates of BGC. However, in a highly selfing species, the overall reduction in BGC should result in a weaker relation. Consistent with this, recombination rate and GC content are not positively correlated in *A. thaliana*, as they are in other genomes (Marais et al. 2004). In the future, it will be important to test for such a correlation using the complete genomes of related selfing and outcrossing taxa.

### Cytoplasmic Male Sterility

Because of the predominant maternal inheritance of mitochondria and chloroplasts, cytoplasmic mutations reducing male fertility will be selectively favored in these genomes if they cause even a slight increase in female fitness, even if total reproductive output is reduced. However, increased female frequencies will often favor the spread of nuclear restorers suppressing male sterility. CMS is a maternally inherited condition leading to male infertility as a result of an inability to produce viable pollen. CMS has been documented in more than 150 plant species and can arise spontaneously in natural populations or after interspecific hybridization (Schnable et al. 1998). Although theoretical work on the population dynamics of plant cytonuclear conflict has focused on gynodioecious species (reviewed in Saur Jacobs and Wade 2003), any outcrossing hermaphroditic species is potentially susceptible to mitochondrial mutations that increase female fertility at a cost to pollen fertility. Indeed, the discovery of CMS in many interspecific crosses is suggestive of a hidden history of cytonuclear coevolution. With high selfing, the selective advantage for pollen sterility mutations in the cytoplasm disappears because CMS mutants reduce female fertility as well. Because of this, we expect much less selection on cytonuclear interactions in highly selfing species, and thus the spread of fitness-reducing pollen sterility mutations should be reduced or eliminated.

Theoretical models of gynodioecy (reviewed in Saur Jacobs and Wade 2003) suggest that fixation of both CMS and restorer alleles is a common evolutionary outcome, with fixation of the restorer allele bringing the population back to hermaphroditism. Evidence for the exposure of "cryptic" CMS through interspecific crosses of hermaphroditic plants is consistent with this hypothesis. If distinct restorer and CMS types have been fixed in different species or populations, wide crosses reexpose this evolutionary history. There are several conditions under which CMS and non-CMS (or restored and unrestored) individuals may be maintained in a population, leading to stable gynodioecy, including pollen limitation experienced by females as they increase in frequency and a fitness cost of the restorer allele (Budar et al. 2003). However, historical "epidemics" of CMS and restoration may be the common outcome, and outcrossing hermaphrodites may have a hidden history of CMS-restorer evolution.

In interspecific crosses, cryptic CMS should be exposed when the maternal parent is outcrossing but not when it is highly selfing. Recently, Fishman and Willis (2006) report cryptic CMS revealed in *Mimulus guttatus* (an outcrosser) × *Mimulus nasutus* (highly selfing) hybrids. They found that hybrid sterility was differentially expressed in the *M. guttatus* cytoplasmic background and that pollenless anther phenotypes were recovered in $F_2$ hybrids with *M. guttatus* cytoplasm but not in the reciprocal hybrids. The lack of CMS phenotypes in the reciprocal $F_2$ hybrids is consistent with predictions from theoretical models because we would not expect to find any evidence for a CMS mutant in a highly inbreeding species. Does cryptic CMS exposed in interspecific crosses generally derive from highly or partially outcrossing species, as opposed to highly selfing taxa? This question is difficult to address because of the limited numbers of species for which quantitative estimates of outcrossing rate are available. It would be interesting to systematically examine pairs of closely related taxa with contrasting selfing rates to test for the degree to which CMS is exposed in interspecific crosses.

Because CMS most often results from novel chimeric mitochondrial proteins (Delph et al. 2007), we might expect the rate of mitochondrial genome structure evolution to be elevated in outcrossers compared to selfers. In addition, CMS-restorer dynamics may lead to a proliferation of members of the pentatricopeptide repeat (PPR) gene family acting as restorers in the nuclear genome. Evidence from genetic mapping of fertility restorers indicates that novel restorers often represent new or mutated forms of a multigene family of PPR genes (Brown et al. 2003; Wang et al. 2006).

In addition to CMS dynamics, antagonistic coevolution between the sex functions should generally be reduced in highly selfing populations as a result of the absence of conflict when the maternal and paternal parents are the same. Brandvain and Haig (2005) hypothesize that this effect will tend to cause asymmetric hybridization success because sexual conflict will tend to lead to growth suppression of offspring by the maternal parent and promote offspring growth by the paternal parent. The net effect in interspecific hybrids is that crosses will be more successful when the selfing species is the maternal parent than in the reciprocal case because sexual conflict is reduced in the inbreeder. A review of the literature generally supports this model and suggests that genomic imprinting driven by sexual conflict has declined in selfing species (Brandvain and Haig 2005).

## Evolution of Genome Size

There is more than 1200-fold variation in nuclear DNA content (C value) in the angiosperms alone. A variety of phenotypic traits have been shown to be associated with C value, including cellular characteristics such as nucleus and cell size (Mirsky and Ris 1951; Price et al. 1973), duration of cell division (Van't Hof and Sparrow 1963; Van't Hof 1965; Bennett 1972), seed size (Beaulieu et al. 2007), and annual or biennial life form (Bennett 1972; Vinogradov 2001). It is possible that genome size has direct effects on these traits and is selected in association with these phenotypes. On the other hand, an alternative class of explanation invokes genetic drift as the primary determinant of genome size. Species with small effective population sizes will experience reduced efficacy of natural selection, leading to the accumulation of slightly deleterious insertions, resulting in increased genome size (Lynch and Conery 2003). Finally, genome size evolution may reflect the selective dynamics associated with TE evolution and other genomic conflicts and/or differences among species in the amount and pattern of DNA repair.

In highly selfing species, genome loss may predominate. Proposed evolutionary forces predicted to reduce genome size in selfers include mechanistic explanations, such as a lower accumulation of TEs and other selfish genetic elements or increased fixation of large underdominant deletions as a result of higher homozygosity in selfers (Charlesworth 1992). In addition, faster cell replication and generation time, both of which are negatively associated with C value, may be selected for in selfing species, leading to an association between selfing and smaller genomes. Annuals tend to have reduced genome size (Bennett 1972), so this would confound effects of selfing and life history because annuals often display higher rates of selfing than perennials (Barrett et al. 1996). Alternatively, if increased genome size is driven by reduced effective population size (Lynch and Conery 2003) or if relaxation of selection against ectopic recombination between TEs drives accumulation in selfers, we would expect increased genome size in selfing species.

There is preliminary evidence that mating system may have an effect on genome size evolution. In *Veronica*, selfers show a significant reduction in genome size, with mating system showing a stronger effect than between annual and perennial sister taxa (Albach and Greilhuber 2004). In their analysis of B chromosomes and genome size, Trivers et al. (2004) also found a strong positive correlation between outcrossing and genome size among plants in the United Kingdom (correcting for B chromosome presence/absence), although this effect was lost when they used phylogenetically independent contrasts.

A limitation of many comparative studies in relation to mating system is the lack of quantitative estimates of selfing rate. Many comparisons rely on descriptions from floras, usually based on floral morphology. Because most models predict a strong effect of inbreeding only with very high selfing rates, analyses based on morphological inferences could mask effects of mating system transitions. Here, we reexplore the effect of inbreeding on genome size by presenting a comparison of C value and genome size in pairs of outcrossing and highly selfing congeners. We refer to DNA amount in a postreplication nucleus; this is equivalent to the 4C DNA amount (C value). However, it is important to distinguish this measure from genome size, defined as the amount of DNA in a monoploid chromosome set. We therefore report genome size as the 4C DNA amount divided by the ploidy of the sample, consistent with the rationale provided by Bennett et al. (1998). A species was considered highly selfing if the outcrossing rate was less than 10% ($t_m < 0.10$). We combined two databases of outcrossing rate from Barrett and Eckert (1990) and modified by Igic and Kohn (2006) with the Kew C value database (http://www.rbgkew.org.uk/cval) and selected those species for which we had both a C value and an outcrossing estimate or evidence of self-incompatibility. We then identified pairs of congeners with contrasting mating systems. From this, there were 14 pairs from nine genera representing eight families, including both eudicots and monocots. A sign test was used to test the prediction that predominantly selfing species have smaller genomes.

While there was no significant pattern of C value reduction in selfers uncorrected for ploidy, there was a significant reduction in genome size in selfing species (fig. 3). This pattern is due to four pairs in which the selfer is polyploid and therefore
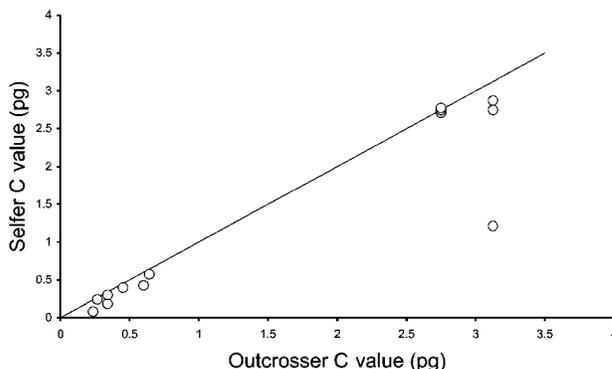


**Fig. 3** C value comparisons for pairs of outcrossing (*X*-axis) and selfing (*Y*-axis) congeners. Each point represents the genome sizes for a single pair of congeners estimated as the 4C value divided by the ploidy. The line is the 1 : 1 line of equality. Note that *Bromus arvenis* and *Hordeum bulbosum* are used as the outcrossing species in comparisons with all selfing congeners in their respective genera. Species pairs are listed with the outcrosser, followed by the corresponding selfer and their respective genome size (in pg): *Amaranthus cruentus* (1.06), *Amaranthus hypochondriacus* (0.96); *Arabidopsis lyrata* (0.94), *Arabidopsis thaliana* (0.32); *Bromus arvensis* (12.5), *Bromus japonicus* (11); *Bromus rubens* (4.85); *Bromus squarrosus* (11.5); *Cerastium arvense* (1.36), *C. arvense fontanum* (0.73); *Glycine argyrea* (2.56), *Glycine soja* (2.3); *Hordeum bulbosum* (11), *Hordeum jubatum* (10.8); *Hordeum spontaneum* (11), *Hordeum vulgare* (11.1); *Phaseolus coccineus* (1.36), *Phaseolus vulgaris* (1.2); *Plantago lanceolata* (2.4), *Plantago major* (1.7); *Senecio squalidus* (1.8), *Senecio vulgaris* (1.58). Genome size data from the Kew C value database (Leitch and Bennett 2004; Angiosperm DNA C value database, http://www.rbgkew.org.uk/cval, ver. 5.0, Dec. 2004). Information on mating systems of species from Barrett and Eckert (1990), modified by Igic and Kohn (2006).

has a much higher DNA amount. In all four of these pairs, the actual genome size is reduced in the polyploid selfer, and there is a significant reduction in genome size across all pairs after controlling for ploidy (sign test, $P = 0.006$). This is consistent with a reduction in genome size in response to either polyploidization or selfing or both. Previous work has shown a general reduction in genome size in polyploids (Leitch and Bennett 2004), likely reflecting DNA loss following whole-genome duplication (Ku et al. 2000). If we exclude pairs with polyploid selfers from the analysis, both C value and genome size are significantly reduced in selfers, suggesting a reduction in predominantly selfing species, independent of ploidal level ($P = 0.016$). Further, after excluding those pairs with annual and perennial congeners, the pattern of reduced genome size in selfers remains significant ($P = 0.016$).

A weakness of our analysis is that in the contrasts involving monocots, two outcrossing species were used repeatedly in comparisons with different selfing species. If selfing evolved independently in each lineage, these should represent independent contrasts. If the selfing species have a shared evolutionary history, the analysis will introduce nonindependence. However, if we take the average contrasts for these groups as single data points, there is still a significant reduction in genome size in the selfing species in our data ($P < 0.01$).

These preliminary results are consistent with a reduction in genome size in self-fertilizing species, but they should be

interpreted with caution because of the small sample size. Nevertheless, this is the first confirmation of a reduced genome size in selfers using taxonomically paired contrasts. It suggests that changes in selection associated with genomic conflict and/or life-history evolution may be more significant for driving genome evolution in selfers than reduced effective population size. The fact that there are four polyploid selfers out of the 14 contrasts may indicate that selection against increased DNA amount is not driving the pattern. Instead, changes to the monoploid genome size, such as a reduction in TEs, are possibly driving this trend. Also, the general association between polyploidy and selfing (see Husband et al. 2008) may have obscured the correlation between selfing rate and genome size in previous work, particularly if some plants in the C value database were incorrectly assumed to be diploid. The role of inbreeding in genome size evolution deserves more thorough investigation as more data become available, but to date, the results are consistent with the prediction of increased fitness and reduced deleterious mutation rates via genomic conflict in inbreeding taxa.

## Conclusions and Future Directions

The preliminary evidence suggests a role for mating system differences in genome evolution. However, the relation between mating system transitions to selfing and reduced fitness associated with the harmful effects of linkage is not clear. Highly inbred taxa may, in some cases, avoid deleterious mutation accumulation through purging of deleterious mutations, large population sizes, the evolution of higher recombination rates, and the elimination of selfish genetic elements. Although there have been few rigorous comparative or experimental tests of the predictions and patterns outlined in this article, future investigations should become increasingly tractable. Completion of the genome of *Arabidposis lyrata*, along with that of the outgroup *Capsella rubella*, will present the first opportunity for large-scale comparison of substitution patterns and genome evolution between closely related species (*Arabidopsis thaliana* vs. *A. lyrata*) with contrasting mating systems. If molecular evolutionary rates and genome structure in these species are examined on a whole-genome scale, there will be extraordinary power to detect differences among species.

Although whole-genome analysis in *Arabidopsis* will be informative, taxonomic replication and a broader sampling of species, particularly using ecological model systems, will be crucial for robust generalizations to be made. This is because studies involving a single comparison are confounded with the history of the lineages involved. Despite independent evolutionary and coalescent history of genes across the *Arabidopsis* genome, providing a form of evolutionary replication,

the entire genome has been influenced by the evolutionary and demographic history of the species sampled. Recent evidence suggests historical bottlenecks in some populations of *A. lyrata* (Wright et al. 2003*b*; Ramos-Onsins et al. 2004), large species-wide effective population size in *A. thaliana* (Nordborg et al. 2005), and the recent and potentially independent evolution of selfing from standing variation in *A. thaliana* (Nasrallah et al. 2004). Collectively, these all point to a complicated demographic history that may reduce the equivalence of the taxa. More widespread taxonomic replication would allow for a detailed picture of the role of mating system evolution and its interaction with ecology and demography.

If many mating system transitions have been recent, especially in short-lived herbaceous taxa, we will need a better theoretical understanding of the timescales and dynamics of genomic changes associated with transition to selfing. Nearly all of the theory discussed in this article assumes selfing populations at long-term equilibrium, and models have not considered the transition to selfing. Such theoretical work will be important in understanding the factors determining the success and/or extinction of selfing lineages. Species in which there is evidence for multiple independent shifts from outcrossing to selfing provide opportunities to examine the genomic consequences of mating system transitions and enable investigation of the interaction of mating patterns and demographic factors (e.g., *Amsinckia spectabilis* [Schoen et al. 1997] and *Eichhornia paniculata* [Husband and Barrett 1993]). Polymorphism and divergence data should also be integrated with genetic information on population structure, gene flow, effective population size, and phylogeographic history. It is critical to consider these effects because while mating system alone can influence effective population size through the processes outlined in our review, diverse ecological factors can also play a role in shaping population genetic structure. A more comprehensive understanding of genome evolution in plants will require information on the interactions among demography, life history, and mating system and how these govern genetic parameters.

## Acknowledgments

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