The Ecological and Evolutionary Consequences of Clonality for Plant Mating

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Abstract
Many flowering plants exhibit dual reproductive modes, producing both sexual and asexual offspring. The commonest form of asexual reproduction is clonal growth, in which vegetative modules (ramets) are produced by the parental genotype (genet). In plants, sexual and asexual reproduction usually occur simultaneously, and this can lead to allocation trade-offs and antagonism between reproductive modes. Our review considers the ecological and evolutionary consequences of functional interactions between clonal reproduction and pollination and mating. Clonal reproduction is commonly associated with mass flowering, restricted pollen dispersal, and geitonogamous self-pollination, processes that can result in inbreeding depression and pollen discounting. We review evidence for the correlated evolution of clonality and sexual systems, particularly self-incompatibility, and identify several floral mechanisms that function to reduce mating costs by limiting selfing and pollen discounting. We conclude by discussing the loss of sexuality in clonal plants and consider the genetic and environmental basis of sexual dysfunction.
1. INTRODUCTION

Reproduction can be conveniently divided into two fundamentally distinct modes—sexual and asexual reproduction—controlled by distinct mechanisms and with contrasting genetic consequences. The evolution of sexual reproduction from asexual reproduction represents one of the major evolutionary transitions in the history of life (Maynard Smith & Szathmary 1995), and understanding the evolutionary maintenance of sex and its costs and benefits has been an enduring problem in evolutionary biology (Otto 2009). Although some organisms reproduce by only one mode of reproduction (e.g., bdelloid rotifers through asexual reproduction and primates by sexual reproduction), a significant proportion of the world’s biota exhibit dual modes, producing both sexual and asexual offspring. Flowering plants (angiosperms) offer unsurpassed opportunities for investigating functional interactions between sexual and asexual reproduction because of their reproductive versatility and experimental tractability.

Flowering plants display enormous diversity in reproductive systems, including many species that combine sexual reproduction with some form of asexual reproduction (Fryxell 1957). The most common type of asexual reproduction is vegetative reproduction, also referred to as clonal growth or vegetative propagation, and it has been estimated that ~80% of angiosperms reproduce by this means (Klims et al. 1997). Clonal growth results in the multiplication of vegetative parts (modules) and occurs in perennial species, many of which dominate major vegetation types on Earth (e.g., grasslands, alpine, tundra, wetland, and aquatic ecosystems). Clonal propagation is also used widely in horticulture, agriculture, and forestry to replicate desirable varieties of domesticated cultivars and produce uniform performance.

Reproduction by vegetative means has several ecological advantages, including persistence in habitats unfavorable for sexual reproduction, the ability of clones to forage for resources in heterogeneous environments, opportunities to spread the risk of death among ramets, and the facility for varying degrees of integration and division of labor among clonal modules (Caraco & Kelly 1991, Silander 1985, Stuefer et al. 1996). A plant’s ability to reproduce by sexual and asexual means also provides alternate routes for genes to be transmitted to subsequent generations. Although possessing dual reproductive modes has clear advantages for perennial species (Silander 1985), these two activities can also interfere with one another, resulting in various forms of antagonism. One of the objectives of this review is to consider how plant fitness is affected by these functional interactions by considering two main sources of antagonism: trade-offs between sexual and vegetative reproduction and the intrusion of vegetative expansion on mating opportunities.

The primary outcome of clonal growth is an increase in the size of individual genotypes. As plants grow they capture resources, occupy more physical space, and, depending on their growth strategy, interact with a larger number of conspecifics and heterospecifics. In species with iteroparous (polycarpic) life histories and indeterminate growth, clonal expansion should lead to more flowering shoots and a greater reproductive capacity. Thus, increased size resulting from clonal growth can be expected to have net fitness benefits because of enhanced fertility. However, increased investment in clonal growth and large clone size can set the stage for functional antagonism between the reproductive activities of clones. The balance between these positive and negative effects of clone size ultimately regulates overall plant fitness.

In contrast to many animal species, in which environmental cues often regulate switches between sexual and asexual reproduction (e.g., cyclical parthenogenesis in some cladocerans, rotifers, and wasps; Bell 1982), these reproductive modes usually occur simultaneously in plants. This raises the question of how sexual and vegetative reproduction interact to determine fitness, and how selection might resolve conflicts between them. One of these conflicts involves allocation trade-offs between sexual and asexual reproduction that limit fitness gains through either reproductive mode.
Clonality is a form of plant growth that results in the production of genetically identical individuals capable of independent growth and reproduction. New individuals formed by clonal propagation are referred to as ramets, and all ramets produced by a genotype are collectively referred to as a genet (Harper 1977). Genets arise from the union of gametes resulting in the formation of a zygote and pass through a unicellular stage, whereas ramets are formed directly from meristematic tissues. In this review, we focus on clonal reproduction in angiosperms, although many of the conclusions we reach also apply to other terrestrial plant groups with clonal growth such as bryophytes and pteridophytes.

Clonality shares some characteristics with vegetative growth because both occur largely through the replication of modular units, e.g., stems and leaves, and to a lesser extent by an increase in the size of existing modules. However, clonality differs from simple plant growth in three important ways. First, ramets resulting from vegetative reproduction are capable of independent growth, including further clonal propagation, and thus clonality has the potential to result in multiplicative growth (Watson 1984). Second, ramets in clonal plants are formed from specialized organs that function, in part, to propagate clonal offspring, including rhizomes, stolons, corms, bulbs, bulbils, and root and stem suckers (Klimeš et al. 1997). Finally, as already noted, clonality provides an alternative to sexual reproduction for the transmission of genes to offspring.

2. TRADE-OFFS BETWEEN CLONAL PROPAGATION AND SEXUAL REPRODUCTION

The balance between sexual reproduction and clonal propagation is regulated by trade-offs that occur either directly, when clonal organs replace sexual structures (e.g., via the formation of
inflorescence bulbils; Ronsheim & Bever 2000), or indirectly via the allocation of resources to either reproductive mode from a common pool of resources. Resource-allocation trade-offs are particularly widespread and have been detected at the ramet level in many clonal species with different modes of vegetative growth. Studies investigating ramet-level trade-offs are of two major types: (a) phenotypic correlations (e.g., Cheplick 1995, Geber et al. 1992); and (b) experimental manipulations of investment in sexual versus clonal reproduction (e.g., Thompson & Eckert 2004, and see van Kleunen et al. 2002). The majority of these studies have detected trade-offs between sexual and clonal reproduction at the ramet level (that is, between these competing functions within shoots).

A key question remains: Do trade-offs detected at the ramet level scale up to the genet level? Unfortunately, few studies have addressed this question even though this is the scale at which selection operates on the evolution of life histories. Significantly, most studies conducted at this level have failed to detect trade-offs between reproductive modes (e.g., Cain & Damman 1997, Mendoza & Franco 1998, Thiele et al. 2009). Although a trade-off between inflorescence and ramet production was detected in clones of Eichhornia crassipes (Geber et al. 1992), there was no evidence for a resource-based trade-off (see also Watson 1984). Instead of resources, the production of inflorescences may limit the availability of meristems used for clonal expansion, thus reducing the rate of genet growth (Geber et al. 1992). Although the formation of clones must entail initial resource costs, these may be small in relation to the ability of new ramets to acquire and contribute resources to the genet.

Interpretation of the effects of trade-offs on genet fitness is further complicated by the fact that a given investment of resources to sexual reproduction may not provide equal fitness returns through investment in clonality. Most angiosperms are simultaneous hermaphrodites, and sex allocation theory predicts that hermaphrodites should have diminishing returns to fitness gains with investment in sexual reproduction (that is, gain curves through female and male sex function are decelerating; Charnov 1979). Diminishing returns might favor the diversion of resources away from sexual reproduction toward clonal propagation. For example, the fitness returns on investment in clonal propagation might often be less strongly diminishing than for sexual reproduction, particularly in habitats in which the survival of clonal offspring is higher than that of sexual offspring (e.g., Prati & Schmid 2000). Unequal fitness returns between sexual and clonal propagation
are implicated in affecting the balance between sexual versus clonal reproduction in habitats over time (Olivieri & Gouyon 1997, Piquot et al. 1998, Silvertown 2008).

Diminishing returns from investment in sexual reproduction could have another effect on the evolution of clonal plant life histories. Under diminishing returns, fitness gains per unit of investment are maximized when ramets have a small investment in sexual reproduction (Zhang 2006). If mating opportunities for each ramet are independent, the diversion of resources from sexual reproduction to clonality could increase total fitness returns through sex in comparison to plants investing solely in sexual reproduction because each ramet would take advantage of the steepest part of the gain curve (Figure 1). This scenario needs to be balanced against the effects of clonal expansion on interference with mating opportunities (see below); however, it highlights
the important point that reproductive trade-offs within ramets are unlikely to scale in a simple
manner to those occurring at the genet level.

3. CLONAL ARCHITECTURE, GENETIC DIVERSITY,
AND SPATIAL GENETIC STRUCTURE

The most obvious consequence of clonality for plant populations is that the number of ramets in
a population does not reflect the number of genets it contains. The relationship between these
two measures provides an index of the extent of clonal propagation in a population. There are
several methods for estimating genotypic (genet) diversity in clonal plants (reviewed in Arnaud-
Haond et al. 2007). The most widely used is the ratio between genets ($G$), usually identified as
unique multilocus genotypes by genetic markers, and the total number of ramets sampled ($N$),
($G/N$ or proportion distinguishable; Ellstrand & Roose 1987). The extent of clonality among
species with diverse modes of asexual propagation has been compiled in several literature surveys
and the estimated value of $G/N$ varies between 0.17 (Ellstrand & Roose 1987) and 0.47 (Arnaud-
Haond et al. 2007). A recent study, excluding apomictic species and trees, has estimated a value of
$G/N = 0.4$ ($n = 77$ taxa, Honnay & Jacquemyn 2008). Using data from studies by Arnaud-
Haond et al. (2007) and Silvertown (2008), and excluding populations of species that are apomictic
or exclusively asexual, we obtained an average value of genotypic diversity for clonal plants of
$G/N = 0.42 \pm 0.02$ (mean $\pm$ SE, $n = 195$ taxa).

The above surveys indicate that variation in genotypic diversity among clonal plant populations
is considerable. Some populations are composed of a single clone, whereas in others each ramet has
a unique multilocus genotype. The values estimated for genotypic diversity indicate that for the
average population of a clonal plant, approximately 4 in 10 ramets sampled are from genotypically
distinct genets. However, this is likely to be an underestimate of the extent of clonality because
ramet sampling is rarely exhaustive and is often designed to maximize the number of genets
sampled. Together with the size of individual ramets, the number of ramets per genet determines
overall clone size. From the perspective of mating, large clone sizes, inferred from low values of
genotypic diversity within a population, result in an increased probability that gametes drawn at
random will belong to the same genet with important implications for mating patterns.

The distributions of clone size within populations of most species are leptokurtic and skewed
to lower size classes. The majority of clones are represented by a few ramets, while some can be
composed of hundreds to many thousands of ramets. This leptokurtic distribution holds regardless
of whether clone size is measured as the number of ramets (Burke et al. 2000, Ruggiero et al. 2005)
or the linear distance between identical genotypes (Peakall & Beattie 1991, Alberto et al. 2005).
The leptokurtic distribution of clone size implies that a few genets disproportionately contribute
to the pollen pool available for mating.

3.1. Spatial Genetic Structure

In addition to clone size, the effect of clonality on mating opportunities is further influenced by the
spatial arrangement of ramets within a clone, referred to as clonal architecture. Plants that clone
by organs that are not easily dispersed, or that are produced relatively close to the parent plant
(e.g., tubers, corms, or tillers in grasses), should have high levels of clonal aggregation (Figure 2).
In contrast, plants cloning via runners, bulbils, or floating vegetative propagules are likely to have
much less clonal aggregation. Clonal architecture is often characterized by the spatial distribution
of ramets produced by a clone with species possessing phalanx (close aggregation of ramets that
exclude other genets) or guerrilla strategies (characterized by rapid spread and a higher degree

Intermingling of genotypes

High
Low
Figure 2
Organs of clonal growth and their potential effect on the degree of intermingling of genets. Phalanx and guerrilla strategies represent two extremes of ramet separation within a genet, which in turn is expected to affect the intermingling of different genets. The order in which we suggest that organs of clonal growth are associated with the production of daughter ramets, which are close to or further away from the parent plant, is for illustrative purposes only, and there are many exceptions. However, in general, structures such as bulbils, which are subject to secondary dispersal from the parent plant, should typically be associated with greater intermingling of clones versus underground structures like rhizomes and bulbs, for which secondary dispersal is less likely. Also, for a given organ of clonal growth, secondary dispersal of daughter ramets is more likely in aquatic rather than in terrestrial environments, increasing the degree of clonal intermingling.

Clonal architecture can be evaluated directly by uncovering connections between ramets (for plants that maintain permanent connections between ramets; e.g., Maddox et al. 1989), or by using genetic markers (e.g., Albert et al. 2008, Aspinwall & Christian 1992). Clonal architecture can also be investigated indirectly by examining the spatial genetic structure (SGS) of populations. Plants that reproduce primarily by vegetative means are expected to show higher SGS than plants with a greater reliance on seed production due to the generally lower dispersal distances of vegetative propagules compared with seeds (Heywood 1991). However, determining the contribution of clonality to SGS requires distinguishing this component from those arising from other aspects of the biology of populations including life form, pollen and seed dispersal, and mating system (Vekemans & Hardy 2004). This can be undertaken by comparing measures of SGS calculated separately at the ramet and genet level because differences between the two should reflect the contribution of clonal growth to SGS. Several methods have been proposed for comparing SGS at the ramet and genet level (Alberto et al. 2005, Procaccini et al. 2007). The few studies that have compared ramet- and genet-level estimates suggest that ignoring clonality usually underestimates SGS by a few meters (e.g., Alberto et al. 2005). In the next section, we describe how clone size and architecture combine to affect pollination and mating opportunities in clonal populations.

4. ECOLOGICAL CONSEQUENCES OF CLONALITY FOR POLLINATION AND MATING

Mating in flowering plants requires two sequential processes to occur—pollination and fertilization. We begin by considering the pollination process in clonal plant populations. In
Self-incompatibility: the inability of a fertile hermaphroditic plant to set abundant seed following self-pollination; contrasted with self-compatibility.

hermaphroditic species, self-pollination can involve pollen dispersal at various spatial scales including within flowers (intrafloral self-pollination), between flowers on the same ramet (within-ramet geitonogamy), and between flowers on different ramets (between-ramet geitonogamy). In animal-pollinated species, each of these three forms of self-pollination can involve pollinators, but only geitonogamous pollen transfer requires pollinators (but see Ågren & Schemske 1993). This is because intrafloral self-pollination can be autonomous if sexual organs are in close contact. Because geitonogamous pollen transfer usually involves the same pollen vectors as cross-pollination, an increase in total self-pollination is likely to come at the expense of cross-pollination, particularly in plants with large floral displays (Harder & Barrett 1996). Clonality should always increase overall levels of self-pollination compared to nonclonal species because there is a higher probability that two randomly sampled flowering shoots in a population belong to the same clone. Moreover, the intensity of self-pollination should increase with clone size and there is some evidence that this occurs at the expense of cross-pollen transfer (Mori et al. 2009).

Clonal architecture should influence the likelihood of geitonogamous self-pollination through its effect on the spatial aggregation of flowering ramets. For example, phalanx plants might be subject to higher levels of geitonogamy than guerrilla strategists (Charpentier 2002). There have been no explicit comparative tests of this prediction; however, Albert et al. (2008) reported that within a population of *Vaccinium myrtillus*, patches with more intermingled clones had higher outcrossing rates than patches in which clones were more clumped. The spatial location of flowers and inflorescences within a clone should also influence the quantity and quality of pollen received. For example, flowers on the periphery of large clones should receive greater quantities of outcross pollen than those in the center (Handel 1985, Reusch 2001). This prediction was tested in apple orchards, in which trees of the same variety were vegetatively replicated and grown in clumped blocks equivalent to clones; however, the data failed to show position effects on pollen capture (Routley et al. 2004). Instead, block size (that is, clone size) had a stronger negative effect on pollen dispersal than on pollen receipt from other clones. Although there is evidence that flowers at the periphery of clones produce more seed than those more centrally located (Free 1979, Wang et al. 2005), such effects have yet to be demonstrated for mating patterns.

The contribution of clonal growth to self-pollination may be significant even if clonality increases SGS by a relatively small amount. Pollen dispersal by both biotic and abiotic vectors usually follows a leptokurtic distribution with most pollen dispersed over relatively short distances (Levin & Kerster 1974). Studies of pollinator behavior in clonal plants generally indicate that most movements occur between nearby inflorescences regardless of pollinator group (Peakall & Beattie 1991). Local foraging by pollinators and the spatial aggregation of flowering ramets within clones therefore constitute the primary mechanisms responsible for the inferior pollen dispersal that characterizes many animal-pollinated clonal plants. However, restricted pollen dispersal is not exclusive to biotic pollination and has also been reported from abiotically pollinated clonal plants (Reusch 2001).

4.1. Mating and Fertility Consequences

The quantity and quality of pollen captured by stigmas of clonal plants is a major determinant of the mating system of populations. In self-compatible hermaphrodites, mating patterns and fertility should be directly influenced by the composition of the pollen load, with selfing rates related to the amount of self-pollen captured. Post-pollination mechanisms such as cryptic self-incompatibility or prepotency of outcross pollen may result in higher levels of cross-fertilization than predicted from the composition of the pollen load (Cruzan & Barrett 1996). However, if geitonogamous pollen arrives early during the pollination process and predominates on stigmas, primary selfing...
Pollen discounting: a loss in outcrossed siring success caused by self-pollination

rates are likely to be considerable. In contrast, self-pollination in self-incompatible species has less influence on the mating system of populations but can lead to reduced fertility and results in a wastage of male gametes (e.g., Hasegawa et al. 2009). Below we discuss the potential for clonality to alter mating success and provide selected examples illustrating these patterns.

4.1. Geitonogamous self-fertilization. Empirical investigations of the contribution of clonality to mating patterns as a result of geitonogamy are few despite established methods for quantifying modes of self-fertilization (Schoen & Lloyd 1992). In the best study, Eckert (2000) used genetic markers and floral manipulations to dissect the mating system in a population of Decodon verticillatus. Approximately 30% of the seed produced resulted from self-fertilization, and when this value was broken down into its component parts, 28% of selfed seeds were found to have resulted from between-ramet geitonogamy. Thus, clonality accounted for more than one quarter of the selfing. If clonal growth causes reductions in clonal diversity per unit area, we expect a negative correlation between the selfing rate and clonal diversity. In the self-compatible sea grass Zostera marina, Reusch (2001) reported that outcrossing rates increased with local genotypic diversity; more genotypically diverse populations tended to have higher outcrossing rates, although this relation was not statistically significant. In D. verticillatus, associations between clonal diversity and outcrossing rate depend on the spatial scale at which they are investigated. Among populations, outcrossing rates were not significantly associated with clonal diversity (Eckert & Barrett 1994). However, at a local scale (that is, among neighboring patches within populations), clonal diversity was positively associated with outcrossing rate (Eckert 2000). More studies are needed to determine more precisely the quantitative relations between variation in clone size and mating patterns.

If self-compatible species exhibit seed abortion due to early-acting inbreeding depression (Husband & Schemske 1996), reduced seed fertility in clones experiencing high rates of geitonogamous self-pollination would be predicted. However, there is only indirect support for this expectation. In Asphodelus aestivus, seed set was inversely related to the proportion of inflorescences belonging to the same genet (Schuster et al. 1993). Similarly, in Vaccinium myrtillus and V. vitis-idaea, seed and fruit set increased with distance between the parents used in hand crosses (e.g., Nuortila et al. 2002). A recent study of Aconitum kusnezoffii found that though the seed fertility of ramets decreased with clone size, there was no evidence that this effect resulted from increased selfing in larger clones (Liao et al. 2009). Hand crosses revealed substantial early-acting inbreeding depression in A. kusnezoffii, which could have obscured the effect of increased geitonogamous pollination on the selfing rate (estimated using mature seeds). This study highlights the diverse factors affecting seed fertility, including resources, pollen quantity, and pollen quality limitation (reviewed in Aizen & Harder 2007). Future studies on the potential costs of geitonogamous selfing in clonal plants should determine the mechanisms responsible for observed variation in seed set.

4.1.2. Fertility costs of clonality in self-incompatible species. The fitness costs to male function of an increase in self-pollination due to clonality are expected to be higher in self-incompatible than self-compatible plants. This is because in self-incompatible plants all male gametes transferred between ramets of the same genet are effectively lost from the pollination process, causing complete pollen discounting (Harder & Barrett 1996). In contrast, the cost of an increase in geitonogamous self-pollination in self-incompatible clonal plants through female function depends on the reproductive and ecological context of populations. For example, when receipt of compatible pollen does not limit seed set, because compatible mates are available and pollinators abundant, (that is, no pollen limitation; Aizen & Harder 2007, Knight et al. 2005), self-incompatibility reduces the female costs of geitonogamous pollination by preventing the production of inbred offspring. Under these conditions, maintaining self-incompatibility is advantageous. However, if the receipt
of compatible pollen is limited, then rejection of self-pollen reduces seed set and maintaining self-incompatibility becomes costly (Porcher & Lande 2005, Vallejo-Marín & Uyenoyama 2004). For these reasons, we might expect the fertility costs of maintaining self-incompatibility in clonal plants to be a function of the level of pollen limitation.

The limited evidence available on outcrossed siring success in self-incompatible clonal plants is consistent with the occurrence of significant costs to male function resulting from pollen discounting. In the partially self-incompatible tree Prunus sibirica, siring success was negatively correlated with clonal growth (Mori et al. 2009). Similarly, in Malus × domestica siring success was not associated with clone size perhaps because larger clones suffer higher levels of pollen discounting than smaller clones (Routley et al. 2004). Because no effect of clone size or ramet position within clones was detected in this study, Routley and colleagues concluded that paternal function is more likely to be sensitive to clonal growth than maternal function. In both of these studies the ramets of different genets were not intermixed and explicit comparisons of the mating consequences of contrasting clonal architectures (e.g., phalanx versus guerrilla) have yet to be examined. The mating costs of geitonogamy due to clonal expansion should be less intense in populations with intermingled clones because neighboring ramets are more likely to represent different genets.

Fertility through female function can also be influenced by clonal growth in self-incompatible plants. For example, in the clonal herb Iris pumila, fruit production increases with clone size but the slope of the relation is significantly less than one (Tarasjev 2005). This suggests that larger clones receive proportionately less compatible pollen. Deposition of incompatible (self-) pollen can also reduce seed set as a result of pollen-pistil interference (e.g., via pollen clogging or ovule discounting; reviewed in Barrett 2002a). As might be expected, the diversity of clones in populations of self-incompatible species can influence female fertility by affecting the pool of compatible pollen available for cross fertilization. Fruit or seed set is positively associated with clonal (or morph) diversity among populations of Calystegia collina (Wolfe et al. 2000), Ditthyrea maritima (Aigner 2004), and Nymphaoides indica (Shibayama & Kadono 2007). Extreme examples of the negative effects of clonality on fertility in self-incompatible species involve populations composed of a single clone or incompatibility group (e.g., Aster furcatus [Les et al. 1991], Hymenoxys acaulis var. glabra [DeMauro 1993], Rubus arcticus [Tammisola 1982], Santalum lanceolatum [Warburton et al. 2000]). The notion that clonality has negative consequences for female and male components of fertility has received considerable attention (Charpentier 2002, Honnay & Jacquemyn 2008), but we still have little data on the magnitude of these costs or on how clone size and architecture influence these costs. Greater attention to these topics will help clarify how the balance between sexual and clonal reproduction affects overall fitness.

5. EVOLUTIONARY IMPLICATIONS OF CLONALITY FOR SEXUAL STRATEGIES

Flowering plants display a remarkably diverse array of sexual strategies and mating systems (reviewed in Barrett 2002b). Considerable theoretical work has focused on key genetic and reproductive parameters (e.g., selfing rates, inbreeding depression, pollen discounting, reproductive assurance) that influence the selection of reproductive traits and patterns of mating (reviewed in Goodwillie et al. 2005). However, despite a rich literature on the evolution of plant mating systems, surprisingly little is known about the ecological and life-history contexts in which transitions between reproductive modes occur. We have shown that clonality has the potential to affect important parameters that feature in most selection models of mating-system evolution. In this section, we consider the role that clonality plays in influencing the evolution of reproductive traits, including self-incompatibility and diverse sexual and floral strategies.
5.1. Association between Clonality and Self-Incompatibility

The widespread occurrence of clonality in self-incompatible species raises the question of how it may influence the evolution and maintenance of self-incompatibility systems. Clonality can affect multiple components of reproduction, resulting in conflicting selection pressures on the maintenance of self-incompatibility (Vallejo-Marín & Uyenoyama 2008). In self-incompatible populations, negative frequency-dependent selection promotes the maintenance of a large number of S-alleles within populations (Lawrence 2000). With gametophytic self-incompatibility, clonal reproduction lowers the number of self-incompatibility alleles because of a reduction in the intensity of frequency-dependent selection (Vallejo-Marín & Uyenoyama 2008). Low allele numbers can significantly increase the evolutionary costs of maintaining self-incompatibility (Vallejo-Marín & Uyenoyama 2004). Interestingly, though clonality reduces allele number, Vallejo-Marín & Uyenoyama (2008) demonstrated that the depth of S-allele genealogies remain large, indicating that clonal reproduction increases the age to the most recent common ancestor of common S-alleles, relative to that expected for nonclonal species. Older alleles can accumulate larger genetic loads linked to specific S-alleles (Stone 2004), which may favor the maintenance of self-incompatibility. Moreover, a recent simulation study found that clonality produced an excess of heterozygotes and consequently increased the mutational load at viability loci unlinked to the S-locus (Navascués et al. 2010). Higher mutational loads should result in more intense inbreeding depression favoring the evolutionary maintenance of self-incompatibility. However, the increase in inbreeding depression can be modest in small populations where genetic drift results in a lack of variation at viability loci (Navascués et al. 2010). Collectively, these theoretical studies indicate that various facets of clonality can result in different consequences for the evolutionary dynamics of self-incompatibility, leading to either its maintenance or its loss.

Clonality may also provide a form of reproductive assurance enabling population establishment and persistence with implications for the maintenance of self-incompatibility. The concept of reproductive assurance has traditionally concerned the ability of plants to set seed in the absence of pollinators or mates through autonomous selfing. However, a broader perspective is used by some researchers to include all forms of uniparental reproduction (Baker 1955, Eckert et al. 2006, Vallejo-Marín & O’Brien 2007). In the absence of clonality, reproductive assurance can occur only in self-compatible populations. However with clonality, genotype persistence in environments with limited pollinator service, or an absence of mates, reduces the intensity of selection favoring the breakdown of self-incompatibility to self-compatibility. A comparative study of Solanum species supports the hypothesis that clonality provides reproductive assurance in circumstances unfavorable for mating (Vallejo-Marín & O’Brien 2007). After taking phylogenetic relationships among species into account, Vallejo-Marín & O’Brien found that self-incompatibility and clonality co-occur more often than expected by chance, which is consistent with the hypothesis that clonality provides reproductive assurance in this group.

The comparative analysis of Solanum by Vallejo-Marín & O’Brien (2007) raises the question of whether more general associations occur between clonality and sexual systems in angiosperms. If clonal reproduction provides reproductive assurance, or if the costs of geitonogamy resulting from inbreeding depression are strong, associations between clonality and sexual systems that enforce obligate outbreeding might be expected. However, if clones receive insufficient pollen to fertilize all ovules this could favor the evolution of some degree of selfing. A recent meta-analysis by Honnay & Jacquemyn (2008) investigated whether indices of genotypic diversity in clonal plant populations and growth architecture (phalanx versus guerrilla, see Figure 2) were associated with obligate outcrossing (including both self-incompatibility and dioecy) or self-compatibility. These researchers predicted that if the costs of geitonogamy are greater than those from pollen...
limitation, clonality should be associated with obligate outcrossing. They found lower measures of genotypic diversity in clonal self-incompatible species compared to self-compatible species, a pattern consistent with their prediction. However, they also found no association between sexual systems and clonal growth architecture, contrary to earlier indications that such associations may occur (Silander 1985, Stebbins 1950). From their findings, Honnay & Jacquemyn (2008) argued that the avoidance of geitonogamous selfing has played a significant role in the evolutionary maintenance of self-incompatibility in clonal species.

### 5.2. Joint Evolution of Selfing and Asexual Reproduction

Clonal plants transmit genes to subsequent generations by both sexual and clonal reproduction. Nagylaki (1976) demonstrated that a mutation increasing selfing should have an equivalent advantage to an alternative mutation increasing the rate of asexual propagation. Nagylaki’s model formalized the fact that the number of total gene copies transmitted to the next generation is the same for selfing and clonal reproduction. This similarity between these reproductive modes is widely recognized (e.g., Holsinger 2000, Kondrashov 1985, Lloyd 1980), but rarely have their evolutionary dynamics been considered jointly. Charlesworth (1980) extended Nagylaki’s (1976) model to include the effect of inbreeding depression and examined the evolution of asexuality in a partially selfing population. He showed that the advantage of a mutation for asexuality depends on the selfing rate and the magnitude of inbreeding depression. With high selfing and low inbreeding depression, the fitness advantage of an asexual mutation decreases. In contrast, when inbreeding depression is high, asexual mutations have a fitness advantage over mutations that increase the selfing rate. Our understanding of the correlated evolution of these two main forms of uniparental reproduction in the context of plant life histories is still in its infancy, and further theoretical and comparative work is desirable.

### 5.3. Clonality, Somatic Mutations, and Mating Systems

In comparison with nonclonal plants, clonality should result in higher per-generation mutation rates because of an increased number of somatic mutations (Klekowski 1998). The per-generation somatic mutation rate is expected to be a positive function of the number of mitotic divisions that occur between zygote formation and gamete production (Scofield & Schultz 2006). To the extent that somatic mutations introduce deleterious alleles, the magnitude of inbreeding depression in clonal plants should be higher than in nonclonal plants. This effect may be ameliorated by the greater longevity of clonal plants, which exposes genotypes to more selection events (Morgan et al. 1997) and opportunities for the purging of deleterious alleles (Charlesworth & Charlesworth 1987). However, theory indicates (Muirhead & Lande 1997) that under a wide range of somatic mutation rates and dominance levels, clonality is generally predicted to increase inbreeding depression in comparison with nonclonal species (but see Marriage & Kelly 2009), although empirical evidence on this issue is sparse.

We investigated the relationship between clonality and inbreeding by extending an analysis conducted by Scofield & Schultz (2006), who compiled adult inbreeding coefficients and selfing rates for plant species with different expected somatic mutation rates. Their results indicated that shrubs and trees, which should accumulate more somatic mutations (high-Φ plants), had lower selfing rates and inbreeding coefficients than herbs (low-Φ plants). They suggested that increased somatic mutations in high-Φ plants caused greater inbreeding depression. Because high inbreeding depression prevents deleterious recessive mutations from being purged (selective interference; Lande et al. 1994), selection should favor high outcrossing rates in high-Φ plants. The same
Do clonal herbs behave as high-$\Phi$ organisms? Adult inbreeding coefficient and progeny selfing rate for (a) nonclonal and (b) clonal herbaceous plants. Inbreeding coefficients and selfing rates were obtained for herbaceous plants from the appendix of Scofield & Schultz (2006). Clonal herbs tend to have low adult inbreeding coefficients and low to high selfing rates, similar to high-$\Phi$ trees and shrubs (Scofield & Schultz 2006), whereas nonclonal herbs show a much wider distribution of values. However, the statistical significance of these trends remains to be assessed. Clonality information was obtained from the literature. Two species of Glycine for which clonality information was not available were not included in this figure.

argument can also be made for clonal plants—they should behave as high-$\Phi$ plants and have low inbreeding coefficients in adults and high outcrossing rates. Using the same list of plants in the appendix of Scofield & Schultz's (2006) paper, we classified species according to whether they were able to reproduce clonally or not. We included only herbaceous species to control for the potential confounding effects of the large size of trees and shrubs. Despite the relatively small sample (81 separate estimates for 20 species), we found that clonal herbs behave as high-$\Phi$ plants, exhibiting low to high selfing rates (up to 0.70) but adult inbreeding coefficients not exceeding $F < 0.4$, whereas nonclonal taxa show patterns characteristic of low-$\Phi$ plants (Figure 3). This suggests that clonality may affect the magnitude of inbreeding depression in herbs with consequences for mating-system evolution.

5.4. Evolution of Floral Mechanisms that Reduce Geitonogamy

Clonality can have diverse and sometimes contrasting ecological and evolutionary consequences for pollination and mating. Next, we consider the extent to which these influences may lead to evolutionary changes in floral strategies and sexual systems. Because of the absence of phylogenetic studies that have investigated the sequence of reproductive trait evolution in clonal groups, our discussion is necessarily somewhat speculative. Our ideas are based largely on the proposition that in clonal species with many simultaneously blooming flowers, mechanisms that reduce the mating costs associated with geitonogamy should be favored.

5.4.1. Dichogamy and herkogamy. The mating costs of mass flowering in clonal plants can be reduced by differences in the relative timing of female and male function (dichogamy). Dichogamy can be expressed at various levels of structural organization including the flower, inflorescence, and plant (Lloyd & Webb 1986). Within-flower dichogamy functioning in isolation will have little
Heterostyly: a sexual polymorphism in which populations contain two (distyly) or three (tristyly) morphs differing reciprocally in style length and anther height.

Influence on geitonogamy in a mass-flowering clonal plant. Even highly synchronized within-ramet dichogamy might not prevent substantial amounts of geitonogamous selfing (e.g., Dorken et al. 2002) or pollen discounting (e.g., Harder & Aizen 2004) in clonal plants. However, synchronization of sex function among ramets of a clone could limit inter-ramet geitonogamy. Unfortunately, there is very little empirical data on synchronized dichogamy among the ramets of a clone, and the limited data available suggest that even plants that maintain connections among ramets do not avoid between-ramet geitonogamy (e.g., Aizen & Basilio 1995, Handel 1985). In andromonoecious Aralia hispida, individual ramets exhibit cycles of male and female function as inflorescence units (umbels) flower in an ordered and nonoverlapping sequence, thus limiting within-ramet geitonogamy (Thomson & Barrett 1981). This flowering pattern (synchronized protandry) is also documented from other families that produced umbellate inflorescences [e.g., Apiaceae (Cruden & Hermann-Parker 1977); Alstroemeriaceae (Snow & Grove 1995); Butomaceae (Bhardwaj & Eckert 2001)]. Significantly, in A. hispida there is evidence that ramets within a clone are also synchronized in their sex function so that between-ramet geitonogamy is to some extent reduced (Thomson & Barrett 1981). Cruden (1988) reported synchronization of sex function from 37 diverse angiosperm families, including many rhizomatous perennials that form extensive clones, (e.g., Typha, Sparganium, Scirpus). Future physiological and developmental studies are required to determine the proximate mechanisms causing coordinated patterns of sex function within and among ramets of a clone.

Heterostyly is a form of herkogamy in which populations are reproductively subdivided into two (distyly) or three (tristyly) self- and intramorph incompatible floral morphs with reciprocal placement of anthers and stigmas (reviewed in Barrett 1992). Experimental studies demonstrate that reciprocal herkogamy functions to promote pollen transfer between morphs, thereby reducing pollen wastage on incompatible stigmas (reviewed in Barrett & Shore 2008, Lloyd & Webb 1992). Enhanced pollen transfer is promoted by the spatial segregation of pollen deposited from the different anther levels on the bodies of pollinators (Wolfe & Barrett 1989). Because heterostyly limits intramorph pollen dispersal, geitonogamous pollen discounting is reduced and may promote more effective pollen carryover than in sexually monomorphic species (Harder & Barrett 1996). Many heterostylous species are highly clonal and are characterized by large floral displays (e.g., Decodon, Eichhornia, Nymphoides, Menyanthes, Oxalis). It would be worthwhile to investigate the correlated evolution of these traits to evaluate the hypothesis that in mass flowering clonal plants heterostyly reduces gamete wastage from geitonogamy. Enantiostyly, a similar floral polymorphism characterized by mirror-image flowers, also reduces geitonogamy in a functionally similar manner (Jessen & Barrett 2005). Therefore, both temporal (dichogamy) and structural (herkogamy) floral mechanisms may serve to limit costs associated with geitonogamy in hermaphroditic clonal species.

5.4.2. Dioecy. Geitonogamous pollination caused by clonal expansion is avoided following the transition from hermaphroditism to dioecy because female and male sex organs occur on separate individuals. Darwin (1876, p. 415) was first to point out the association between dioecy and large plant size. Although he studied trees rather than clonal plants, his argument is analogous to the one made here. Theoretical models and empirical evidence indicate that selfing and inbreeding depression play critical roles in the evolution of dioecy (Charlesworth 1999). We have shown above that both of these factors are likely to be exacerbated by clonality and therefore high rates of geitonogamous selfing in the hermaphroditic ancestors of many clonal plant lineages may be an important mechanism favoring the evolution of unisexuality (de Jong & Geritz 2001, Reusch 2001, Thomson & Barrett 1981).
Dioecy is common in groups that rely heavily on clonal propagation, particularly aquatic plants, including marine angiosperms (Les 1988). Associations between large plant size and generalist pollination, including abiotic pollination, are common in dioecious plants (Sakai & Weller 1999), and random and/or restricted pollen dispersal may aggravate any size-related increases in geitonogamy. However, associations between clonality and dioecy have not been rigorously examined in angiosperms (for a comparative analysis of associations between dioecy and asexuality in mosses, see Crawford et al. 2009), and without phylogenetic data such associations are difficult to interpret. For example, dioecy and large clone size might co-occur because either: (a) dioecy alleviates the negative consequences of geitonogamy, enabling the subsequent evolution of large clone size; or (b) extensive geitonogamous selfing in clonal populations favors the separation of the sexes. In the clonal aquatic *Sagittaria latifolia* both hermaphroditic (monoeccious) and dioecious populations occur, and several lines of evidence indicate that dioecy is the derived condition (Dorken & Barrett 2004). Geitonogamous selfing, promoted by extensive clonality, and inbreeding depression are likely factors causing the evolution of dioecy in this species (Dorken et al. 2002). However, because of the absence of comparative data on the sequence in which large clone size and dioecy originated in *Sagittaria*, it is not possible to completely rule out the alternative scenario that large clone sizes followed rather than preceded the evolution of dioecy.

6. LOSS OF SEX

Functional antagonism between sexual and asexual reproductive modes is reduced to zero for clonal plants that abandon sexual reproduction altogether. In most cases where populations reproduce solely by clonal growth, environmental factors (e.g., lack of pollinators, unfavorable conditions for flowering, seed maturation, or seedling establishment) are the main cause of sexual failure. Historical contingency can also result in the disabling of sexual systems when founder events result in the absence of mating partners, which is a pattern common among clonal invasive species (reviewed in Barrett et al. 2008). Asexual reproduction tends to predominate in environments unfavorable for sexual reproduction [e.g., high latitudes and elevations (Salisbury 1942), aquatic environments, (Barrett et al. 1993)]. Under these circumstances, the investment of resources into sexual reproduction may yield minimal fitness returns, and the strength of selection maintaining pollen and ovule fertility is weak, allowing the spread of sterility mutations. Moreover, if there are trade-offs between sexual and clonal reproduction, selection may favor reduced investment in sex and increased allocation to survival and/or clonal propagation. This combination of relaxed selection and trade-offs can promote the evolutionary loss of sex in clonal populations.

Although uncommon, there is evidence for the evolutionary loss of sex in clonal species as a result of genetic factors unrelated to polyploidy or hybrid sterility (reviewed in Eckert 2002). For example, in *Decodon verticillatus* sex has been lost at the northern edge of the species’ range in eastern North America, where populations persist solely by clonal propagation and typically consist of single clonal genotypes (Eckert & Barrett 1993). In this species, sexual sterility is evident under both natural and glasshouse conditions and appears to result from genes with pleiotropic effects on fitness (Dorken et al. 2004). Northern populations of *D. verticillatus* with low sexual fertility have higher overwintering survival under uniform growth conditions. More attention should be given to populations of clonal species at range limits to determine how often genetically based sterility occurs and to what extent limited genetic variation contributes to the establishment of range boundaries.
SUMMARY POINTS

1. Clonal plants display a wide range of growth strategies that influence spatial genetic structure, the degree of aggregation of reproductive units (flowers and inflorescences), and pollen dispersal.

2. There is considerable empirical evidence for fitness trade-offs between sexual and clonal propagation at the ramet but not the genet level, even though this is the scale at which selection operates.

3. Large clones often produce many simultaneously flowering blossoms, promoting considerable between-ramet self-pollination (geitonogamy) and resulting in two primary mating costs: inbreeding depression and pollen discounting.

4. The mating and fertility consequences of clonality are mediated by the sexual system, especially whether an individual is obligately outcrossing or can self-fertilize.

5. Clonality has the potential to influence the evolution of sexual strategies through its effects on allelic diversity, pollen limitation, reproductive assurance, and mutational load.

6. The accumulation of somatic mutations in clonal plants may have the effect of making them behave as high-\( \Phi \) plants, with low adult inbreeding coefficients and high outcrossing rates.

7. In clonal plants, several floral mechanisms including synchronized dichogamy, heterostyly, and dioecy function to limit or prevent mating costs associated with geitonogamous self-pollination.

8. Environmental and genetic factors can result in the loss of sexual reproduction in clonal populations and lead to genetic uniformity, particularly at range limits.

FUTURE ISSUES

1. Incorporate clonal propagation and its reproductive consequences into theoretical models of the evolution of sex allocation, mating patterns, and sexual systems.

2. Use hypervariable genetic markers (e.g., microsatellites) to directly measure variation in clone size in natural populations.

3. Investigate the extent to which clonal and sexual reproduction trade off at the genet level and determine the appropriate currencies involved in such trade-offs.

4. Design experimental studies using genetic markers to determine the extent to which clone size and architecture influence pollen dispersal, mating patterns, and pollen discounting.

5. Conduct sister-group comparisons and intraspecific studies of clonal and nonclonal species and populations, respectively, to investigate differences in inbreeding depression and mating systems.

6. Use comparative and phylogenetic methods to investigate associations between sexual systems (e.g., self-incompatibility and dioecy) and clonality, and determine patterns of correlated evolution and the sequence of origination of traits within lineages.
7. Investigate the extent to which clonal integration may promote genet-wide synchronization of sex phases (synchronized dichogamy) as a mechanism to reduce geitonogamous pollination, and determine the underlying developmental and physiological mechanisms responsible.

8. Compare central and marginal populations of clonal species to investigate variation in reproductive modes and the ecological and genetic factors governing the loss of sex at range limits.

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LITERATURE CITED


Conducted an experimental study of the relation between clonal architecture and the selfing rate.

Reviews methods for genetic analysis of clonal populations and the need for a standardized approach.
Conducted a theoretical study of the evolution of the selfing rate in a partially asexual population.

Reviews how clonal growth strategies may influence pollen dispersal and mating in plant populations.

Conducted an experimental field study using genetic markers in which the contribution of inter-ramet geitonogamy to selfing is measured.


DeMauro MM. 1993. Relationship of breeding system to rarity in the lakeside daisy (*Hymenoxis acanlis var. glabra*). *Conserv. Biol.* 7:542–50


Was the first detailed consideration of how clonal growth influences pollen dispersal, gene flow, and mating patterns in flowering plant populations.

Conducted a meta-analysis of clonal taxa demonstrating that populations of self-incompatible and dioecious taxa have less genetic diversity than self-compatible species.
Salisbury EJ. 1942. The Reproductive Capacity of Plants. London: Bell

Conducted an experimental study using genetic markers, reporting that clonal growth reduces male reproductive success because of pollen discounting.

Conducted a theoretical study of the general consequences of somatic mutations for inbreeding depression in clonal plant populations.


