The loss of sex in clonal plants

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Abstract. Most plants combine sexual and clonal reproduction, and the balance between the two may vary widely between and within species. There are many anecdotal reports of plants that appear to have abandoned sex for clonal reproduction, yet few studies have quantified the degree of sexual variation in clonal plants and fewer still have determined the underlying ecological and/or genetic factors. Recent empirical work has shown that some clonal plants exhibit very wide variation in sexual reproduction that translates into striking variation in genotypic diversity and differentiation of natural populations. Reduced sexual reproduction may be particularly common at the geographical margins of species' ranges. Although seed production and sexual recruitment may often be limited by biotic and abiotic aspects of the environment in marginal populations, genetic factors, including changes in ploidy and sterility mutations, may also play a significant role in causing reduced sexual fertility. Moreover, environmental suppression of sexual recruitment may facilitate the evolution of genetic sterility because natural selection no longer strongly maintains the many traits involved in sex. In addition to the accumulation of 'neutral' sterility mutations in highly clonal populations, the evolution of genetic infertility may be facilitated if sterility is associated with enhanced vegetative growth, clonal propagation or survival through either resource reallocation or pleiotropy. However, there are almost no experimental data with which to distinguish among these possibilities. Ultimately, wide variation in genotypic diversity and gene flow associated with the loss of sex may constrain local adaptation and the evolution of the geographical range limit in clonal plants.

Key words: asexuality, clonal reproduction, genotypic diversity, mutation, peripheral populations, range limits, sexual sterility, trait loss

Introduction

Most perennial plants possess two modes of regeneration: sexual reproduction through seed and clonal reproduction through some form of vegetative propagation (Richards, 1986). The relative importance of sexual vs. clonal recruitment may vary widely among plant species as well as among populations within species. For example, there are many anecdotal reports in the literature of species having abandoned sexual reproduction for some form of clonal reproduction, at least in some habitats or parts of their geographic range (Sculthorpe, 1967; Hutchinson, 1975; Philbrick and Les, 1996).

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Clonal reproduction does not involve recombination and, therefore, yields offspring that are genetically identical to each other and to the plant that produced them. Because clonal offspring are also usually much larger than seed and lack prolonged dormancy or specialized dispersal mechanisms, it is likely that they probably experience reduced dispersal compared to offspring produced through sexual reproduction (Ashton and Mitchell, 1989; Starfinger and Stöcklin, 1996). High levels of clonal recruitment are, therefore, expected to strongly influence genetic variation within and among populations (Silander, 1985; Muirhead and Lande, 1997), effective population size and hence stochastic genetic processes (Gliddon *et al.*, 1987; Eckert and Barrett, 1992, 1995; Orive, 1993), metapopulation dynamics (Olivieri and Gouyon, 1997), the way in which natural selection acts on populations (Vuorisalo *et al.*, 1997), and perhaps even the evolution of the geographic range (Dorken and Eckert, 2001). Yet, there has been little effort to quantify how much the reproductive mode varies within or among clonal plants, identify the ecological and/or genetic factors underlying this variation, and explore the evolutionary causes and consequences of wide variation in sexuality. In this review, I discuss the ecology, genetics and evolution of sexual variation in clonal plants, and will illustrate how key questions might be answered.

Geographic variation in sexual reproduction

The relative importance of sexual vs. clonal recruitment may vary among populations of clonal plants because clonal reproduction allows populations to persist in habitats or regions where, for one reason or another, sexual reproduction cannot occur. For example, the rampant spread of dioecious *Elodea canadensis* (Canada pondweed) across Europe involved only female plants (Sculthorpe, 1967); and male and female plants in *Stratiotes aloides* (water soldier) tend to be confined to different parts of the species' European range so that sex is not possible in most populations (Hutchinson, 1975; more examples in Preston and Croft, 1997).

Flower, fruit or seed production and/or seedling recruitment has also been observed to vary among populations of clonal plants, especially near geographical range boundaries (Salisbury, 1942; Eriksson, 1989, 1996). However, there has been little attempt to quantify variation in sexual reproduction (Barrett, 1980a; McKee and Richards, 1996) or to determine whether reduced sexual reproduction has an impact on long-term sexual recruitment (Aspinwall and Christian, 1992).

Our work on the tristylous, aquatic plant *Decodon verticillatus* revealed a strong link between severely reduced seed production and the failure of sexual recruitment in geographically marginal populations. In the central portion of

its North American range, plants exhibit prodigious seed production as well as vigorous clonal propagation through adventitious rooting of branches (Eckert and Barrett, 1995). Variation in sexual reproduction among populations was first suggested by wide variation in the frequency of the three style morphs among populations. In Figure 1, I compare the pattern of morph frequency variation observed in *D. verticillatus* with that observed in related but predominantly sexual *Lythrum salicaria* (purple loosestrife; Eckert and Barrett, 1992, 1995; Eckert *et al.*, 1996). *Decodon verticillatus* exhibits much wider variation in morph frequencies among populations and a much higher frequency of populations that contain only a single style morph (monomorphic populations). Computer simulations suggested that such a high frequency of monomorphism could only occur if sexual recruitment is severely suppressed in some populations (Eckert and Barrett, 1992). In addition, these monomorphic populations are most common near the northern limit (but not the southern limit) of the species' range (Fig. 2; see also Eckert and Barrett, 1992). An investigation of variation in sexual fertility among 28 populations across a 500-km latitudinal gradient supported this hypothesis by revealing a marked reduction in all components of sexual reproduction, from flower production, through pollen tube growth to seed set, in northern monomorphic compared to
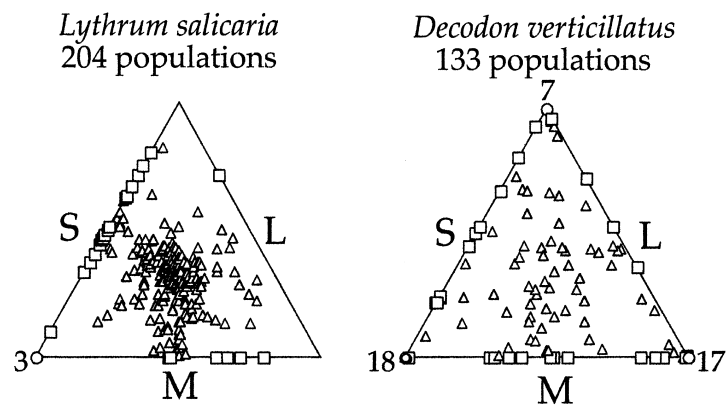

Figure 1. Patterns of variation in style morph frequencies among populations of predominantly sexual *Lythrum salicaria* and predominantly clonal *D. verticillatus*. Each side of the triangle represents one of the three style morphs (L = long-styled, M = mid-styled, S = short-styled). Each point is an individual population, and the frequency of each morph in a population is proportional to the distance of the point from each morph's axis. Populations with equal frequencies of all three morphs lie at the center of the triangle. Those lacking a morph (dimorphic populations) lie on the axis corresponding to the morph lacking. Those consisting of only one morph (monomorphic populations) lie on the vertex opposite to the single morph occurring in that population. Numbers indicate the number of monomorphic populations lying on that vertex. Note the much larger frequency of monomorphic populations in *D. verticillatus* (31.6%) than in *L. salicaria* (1.5%). Most of the monomorphic populations of *D. verticillatus* are located near the northern margin of the geographical range. Data are compiled from Eckert and Barrett (1992, 1995) and Eckert *et al.* (1996).
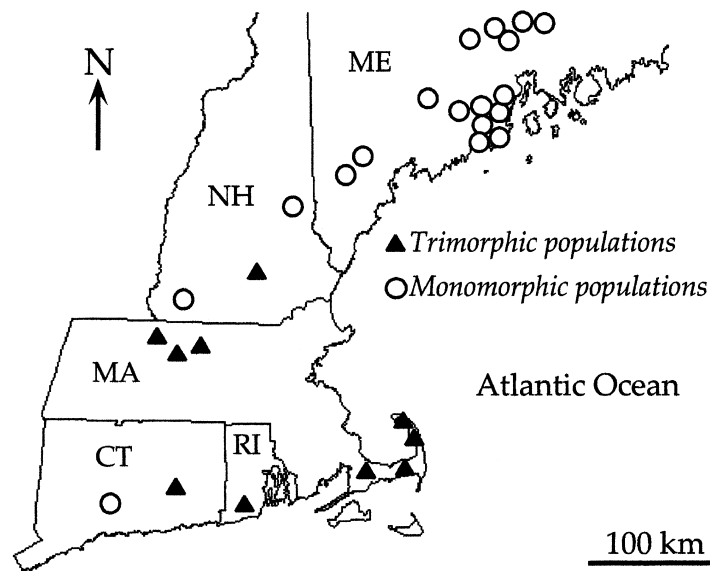
Figure 2. Geographic distribution of florally monomorphic and trimorphic populations of *D. verticillatus* studied in New England, USA. (CT = Connecticut, MA = Massachusetts, ME = Maine, NH = New Hampshire, RI = Rhode Island). Redrawn from Dorken and Eckert (2001). A more comprehensive distribution of populations in New England appears in Eckert and Barrett (1992).

southern trimorphic populations (Fig. 3). On average, ramets in monomorphic populations produced only 15 seeds compared to 1139 in trimorphic populations, and one half of all monomorphic populations produced no seed at all (Dorken and Eckert, 2001).

Long-term sexual recruitment inferred from patterns of genotypic diversity

Whether greatly reduced seed production limits long-term sexual recruitment in clonal plants can be inferred from levels of genotypic diversity within populations. Comparisons of allozyme diversity in northern vs. southern populations of *D. verticillatus* revealed a strong association between seed fertility and genotypic diversity (Eckert and Barrett, 1993; Dorken and Eckert, 2001). Most northern populations consisted of single multilocus genotypes, whereas southern populations were genotypically diverse, with genotype frequencies at Hardy–Weinberg equilibrium. In addition, single-genotype populations were usually fixed for heterozygous genotypes. Most of these massive clonal genotypes are restricted to single populations, suggesting that gene flow is very low between northern populations (see also Aspinwall and Christian, 1992; Piquot *et al.*, 1996).
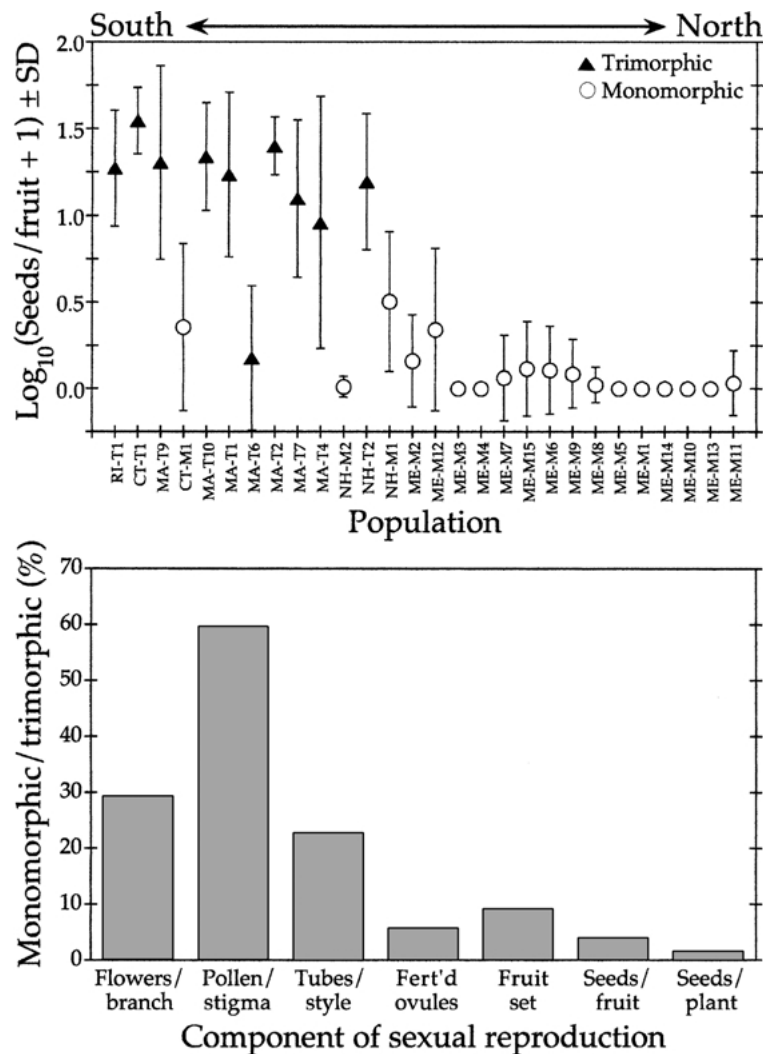
Figure 3. Wide variation in sexual reproduction among populations of *D. verticillatus* near the northern limit of the geographical range in New England. The top panel illustrates the variation in mean ± SD seeds/fruit calculated from a sample of five fruit from each of about 30 plants per population. The bottom panel shows the overall mean of northern monomorphic populations expressed as a percentage of the mean for more southerly trimorphic populations for seven sequential components of sexual reproduction. Data are from Dorken and Eckert (2001).

Estimates of genotypic diversity in clonal populations are sensitive to sampling effort (Hebert *et al.*, 1988; Aspinwall and Christian, 1992; Widén *et al.*, 1994), therefore, conclusions concerning the number of genotypes per populations, and hence the frequency of sexual recruitment would seem to require large

samples of ramets assayed for many marker loci. However, co-dominant genetic markers like allozymes allow the detection of fixed heterozygosity, which strongly suggests that sexual recruitment rarely occurs (Soltis *et al.*, 1988; Piquot *et al.*, 1996). Dorken and Eckert (2001) calculated the detectability of sexual recruitment when fixed heterozygosity is found among small samples of ramets ($n = 12$/population) assayed for few codominant loci ($n = 3$) to show that sexual recruitment rarely, if ever, occurs in northern populations of *D. verticillatus*.

Inferring the relative importance of sexual vs. clonal recruitment from genetic data is much more challenging when there are multiple genotypes per population, as it is usually difficult to distinguish sexual recruitment from immigration of new genotypes. In addition, low levels of sexual recruitment can produce substantial genotypic diversity (Watkinson and Powell, 1993). Techniques that use measures of genotypic relatedness based on highly polymorphic markers (e.g. AFLPs and microsatellites) combined with spatial analyses are likely to provide greater insight into the processes responsible for genotypic diversity in clonal populations (Harada *et al.*, 1997; Lynch and Ritland, 1999; Reusch *et al.*, 1999; Smouse and Peakall, 1999).

Results from *D. verticillatus* seem at odds with two widely cited reviews which argue that clonal plants usually exhibit substantial genotypic diversity within populations (Ellstrand and Roose, 1987; Widén *et al.*, 1994). Broad comparisons of genetic diversity from published studies have also failed to reveal much difference in population genetic differentiation between sexual and predominantly clonal species (Hamrick and Godt, 1990). These studies lead to the impression that clonal reproduction, despite its theoretical consequences, does not greatly influence the genetic structure of natural populations (McLellan *et al.*, 1997). However, the scale of sampling both within and among populations varies tremendously among studies such that significant effects of clonality at any given scale may go undetected. For instance, clonal reproduction may produce significant genotypic patchiness within populations (Reusch *et al.*, 1999) that can have a variety of important consequences, for example substantial levels of geitonogamous self-fertilization in populations of *D. verticillatus* (Eckert, 2000). At a broader scale, most interspecific comparisons have focussed on average levels of genotypic diversity rather than the wide range of diversity that occur across the geographic ranges of clonal species. Data on genotypic diversity in 32 clonal species summarized by Widén *et al.* (1994) indicate that single-genotype populations occur along with genotypically diverse populations in more than one-third of all species (see also Les, 1991; Aspinwall and Christian, 1992; Piquot *et al.*, 1996). As I will discuss below, wide variation in population genetic structure (and presumably sexual recruitment) may have significant evolutionary consequences.

What factors limit sexual recruitment in clonal populations?

There is abundant evidence that ecological factors, both biotic and abiotic, can affect seed production, germination and seedling recruitment in plants (Abrahamson, 1980; Richards, 1986). Sometimes one key factor is involved. For example, many aquatic plants with aerial flowers fail to produce flowers or seeds when growing in deep water (Hutchinson, 1975). Cold temperatures may also single-handedly reduce seed production (e.g. Pigott, 1981; Pigott and Huntley, 1981). However, several factors may interact to constrain the niche space in which sex occurs (Caughley *et al.*, 1988). Observations that seed production or some other component of sexual reproduction is greatly reduced in populations of clonal plants near geographic range limits (Ashton and Mitchell, 1989; Eriksson, 1996) suggest that ecological factors commonly underlie sexual variation in clonal plants. However, genetic factors are also known to cause sexual sterility, and ecological and genetic factors may interact over evolutionary time. Klekowski (1988a, b) hypothesizes that genetic sterility factors may become fixed in clonal populations where sexual recruitment is first hampered by ecological factors (see also Sculthorpe, 1967; Hutchinson, 1975; Barrett, 1980b; Eriksson, 1992; Barrett *et al.*, 1993). Thus, ecological sterility may set the stage for the evolution of genetic sterility (see below).

Transient genetic infertility may arise at the population level when seed production is impaired by low mating type diversity in sexually polymorphic species (Byers and Meagher, 1992; Reinartz and Les, 1994; O'Connell and Eckert, 1999; Charpentier *et al.*, 2000). However, sexual fertility can be fully restored by the immigration of rare mating types. Permanent genetic sterility at the genet level may be caused by a change in ploidy or some other chromosomal irregularity that impairs meiosis (Stebbins, 1971). Triploids and other sterile polyploids are not uncommon among clonal aquatic plants (Les and Philbrick, 1993; Preston and Croft, 1997), and sterile polyploids often occur independently of the parental species that gave rise to them (Hutchinson, 1975) and sometimes become extremely widespread (e.g. *Acorus calamus* in Britain and Europe – Hutchinson, 1975; Preston and Croft, 1997; *Hydrilla verticillata* in Japan – Verkleij *et al.*, 1983; Nakamura *et al.*, 1998; *Oxalis pes-caprae* in North America and Australia – Baker, 1965; *Salvinia molesta* in Africa and India – Ashton and Mitchell, 1989).

Wide variation in sexual fertility among populations within species is sometimes caused by variation in ploidy. For example, seed production covaries strongly with ploidy among populations of the aquatic plant *Butomus umbellatus*. Chromosome counts combined with measures of pollen size and shape (which reliably indicate ploidy in this species) show that diploid populations produce abundant seed whereas triploid populations produce little or

no seed (Fig. 4; see also Krahulcová and Jarolímová, 1993; Eckert *et al.*, 2000). Among introduced populations of *B. umbellatus* in North America, sterile triploid populations tend to occur further north and west than fertile diploid
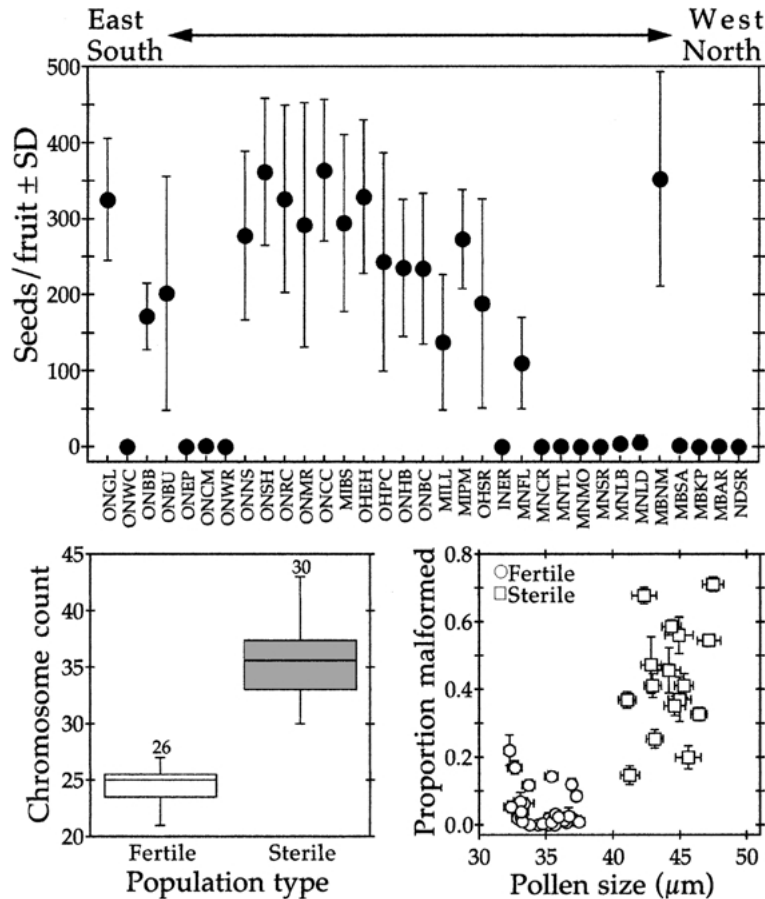

Figure 4. Wide variation in sexual fertility due to variation in ploidy among introduced populations of *B. umbellatus* in North America (K. Lui, F.L. Thompson and C.G. Eckert, unpublished data). The top panel illustrates variation in mean ± SD seeds/fruit among 33 populations based on one fruit sampled from each of 10–48 ramets per population. The populations are distributed along a 2000 km transect running from eastern Ontario (ON) to Minnesota (MN), Manitoba (MB) and North Dakota (ND). The bottom left panel contrasts the distribution of somatic chromosome counts between nine sexually fertile and nine sexually sterile populations. The data are based on two cells from each of three plants per population. The bottom and top of each box plot marks the 25- and 75-percentile, respectively. The midline is the median, and the whiskers extend to the minimum and maximum values. Sterile populations have average counts close to the expected triploid number $3x = 39$ (mean ± SD = $35.6 ± 2.9$), whereas fertile populations have counts close to $2x = 26$ ($24.7 ± 1.4$). The bottom right panel shows that sexual sterility is generally associated with triploidy by contrasting population mean pollen size and proportion of grains exhibiting malformed shape for 44 introduced populations. Error bars are ±1SE.

populations (Fig. 4). This may be an additional consequence of variation in ploidy, as polyploids are often distributed at higher latitudes and altitudes compared to closely related diploids (Bierzychudek, 1987), possibly due to an enhanced tolerance of extreme environments associated with an increase in chromosome number (Levin, 1983).

Genetic sterility may also result from mutations in the nuclear or organellar genomes that impair one or more of the many processes involved in sexual reproduction (Klekowski, 1988a, b). An experiment involving fertile and infertile populations of *D. verticillatus* grown and crossed in a common greenhouse environment revealed that, even under benign greenhouse conditions, plants from infertile populations continued to exhibit greatly reduced seed production compared to those from fertile populations (Fig. 5). This indicates a genetic basis to the low fertility of northern populations exhibited in the field. A more detailed quantitative genetic experiment further showed that genetic sterility may involve nuclear mutations at one or very few loci (Eckert *et al.*, 1999).

Ecological and genetic factors may jointly contribute to reduced sexual fertility in clonal populations, however, the relative importance of genes and environment has rarely been investigated. By comparing the sexual fertility of
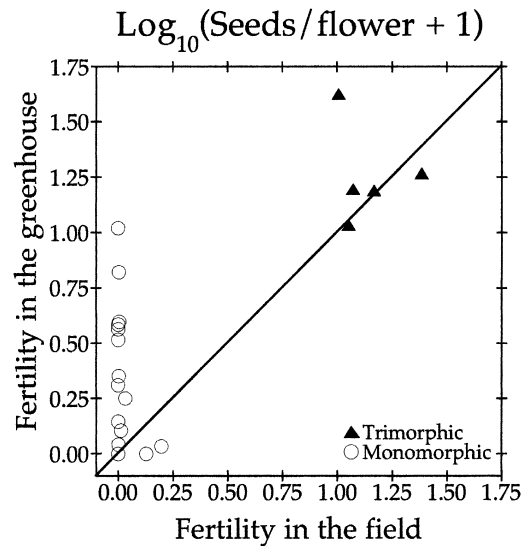

Figure 5. Comparison of population fertility under field conditions and in a common greenhouse environment. Northern monomorphic populations were much less fertile than more southerly trimorphic populations in both environments. However, monomorphic populations exhibited a significant increase in fertility in the greenhouse compared to the field (paired $t = 2.74$, $df = 15$, $p = 0.0152$), whereas trimorphic populations did not ($t = 0.92$, $df = 4$, $p = 0.41$). Field data come from about 30 plants per population. Greenhouse data come from about 15 flowers pollinated on each of about seven plants per population. Data are from Dorken and Eckert (2001).

northern vs. southern population of *D. verticillatus* in the greenhouse with their fertility in natural populations, Dorken and Eckert (2001) showed that, although plants in northern populations are genetically infertile, ecological factors also reduce seed production. Most northern populations had significantly higher seed fertility in the greenhouse than in the field, whereas the fertility of southern populations did not differ between environments (Fig. 5). This suggests that the fertility of northern populations is being dampened by environmental factors in addition to genetic factors. For some populations, the effect of environment was very strong.

The environmental factor(s) that reduce fertility in *D. verticillatus* are, at this point, not known. The strong correlation between population fertility and latitude suggests that the factors responsible probably covary with latitude (Dorken and Eckert, 2001). Temperature seems a likely factor, as it usually correlates negatively with latitude, and is widely thought to be a proximate constraint on the geographical distribution of plant species (Pigott and Huntley, 1981; Woodward, 1990). However, the role of even a relatively simple factor like temperature in influencing the balance between sexual and clonal reproduction is, as yet, poorly understood.

How does genetic sterility evolve?

The prevailing hypothesis for the evolution of genetic infertility is that sex is lost through the accumulation of sterility mutations in populations where sexual recruitment is impaired by the environment (Klekowski, 1988a, b). This stems from the general prediction that traits which no longer enhance fitness should be degraded by mutation (Haldane, 1933; Muller, 1949; Emerson, 1961; Fong *et al.*, 1995), a phenomenon best illustrated by the loss of eyes and pigments in cave-dwelling organisms (Culver, 1982). The 'use it or lose it' hypothesis seems a likely explanation for the loss of sex in *D. verticillatus*. Sexual recruitment in northern populations is impaired by ecological factors, providing conditions for the fixation of sterility mutations (Eckert *et al.*, 1999).

Sexual recruitment, and hence the fitness value of seed production, may also vary with habitat successional stage. For example, sexual recruitment decreases with canopy closure in woodland populations of *Uvularia perfoliata* (Kudoh *et al.*, 1999) and with vegetational maturation of disturbed freshwater wetlands in *Sparganium erectum* (Piquot *et al.*, 1996). However, seed remains the primary vehicle for the recolonization of disturbed habitats, thus new populations should have a preponderance of sexually fertile genotypes. If there is a trade-off between sexual and clonal reproduction, metapopulation dynamics will lead to sexual reproduction being favored during colonization but clonal reproduction being favored as habitat patches mature (Olivieri and Gouyon, 1997).

Piquot *et al.* (1998) supported this hypothesis by comparing components of clonal and sexual reproduction among new and old populations of *Sparganium erectum* (Fig. 6). New populations were more sexually fertile than older populations but exhibited lower levels of clonal ramet production.

The initial loss of sexual reproduction due to genetic factors may set the stage for the mutational degradation of other traits associated with sexual reproduction. For example, sexual traits should become degraded in popula-
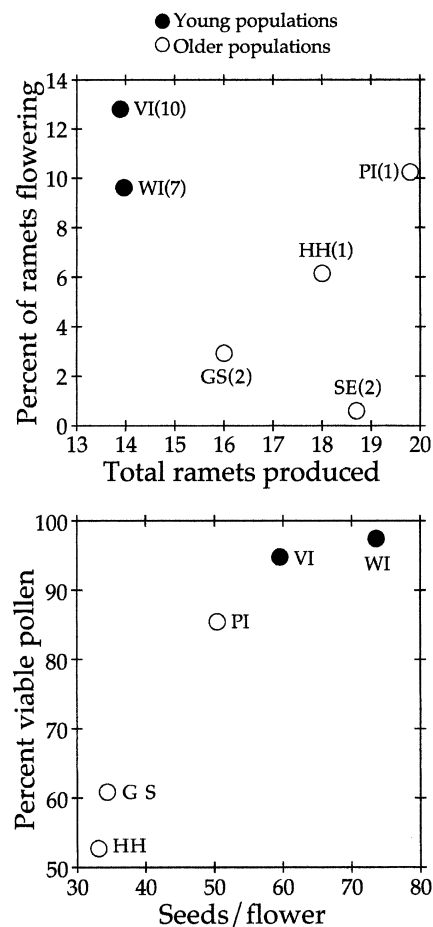

Figure 6. Variation in the capacity for sexual and clonal reproduction between plants from early-successional (young) and late-successional (older) populations of *S. erectum* grown in a common garden environment. Population means are based on three clonal replicates of each of 10 ramets per population grown under mesotrophic conditions for 5 months. Pollen viability was assessed using Alexander's staining method for 200 pollen grains per clonal replicate. Data are from Piquot *et al.* (1998). Each point is identified by population code. Values in brackets are the numbers of distinct genotypes detected among a sample of 10 ramets from each population based on variation in allozymes and oligonucleotide DNA fingerprints.

tions that have been rendered sterile by a change in ploidy. However there has been almost no attempt to determine the evolutionary fate of sexual characters in sterile polyploids (Suda, 1995) even though they have arisen repeatedly in many groups of plants. Sexual traits appear to have degenerated in sterile triploid populations of *B. umbellatus* (Fig. 7). In fertile diploid populations, individual ramets typically produce 2–4 inflorescences over a growing season, whereas plants in sterile triploid populations flower only rarely, if at all. Fertile diploids also exhibit strong temporal separation of anther dehiscence and stigma receptivity within flowers (dichogamy), a floral mechanism thought to facilitate cross-pollination and reduce self-pollination (Lloyd and Webb, 1986). Fertile populations also exhibit synchronous dichogamy so that whole inflorescences go through sequential periods of being unisexual male or unisexual female, which may reduce self-pollination between flowers within inflorescences (Bhardwaj and Eckert, 2001). In contrast, plants in sterile populations exhibit greatly weakened within- and among-flower dichogamy. Diploids and triploids are strongly differentiated from one another at RAPD loci, suggesting that there has been substantial evolutionary time for the degeneration of sexual characters to evolve since the origin of triploidy (K. Lui, P. Corradini, A. Bruneau and C.G. Eckert, unpublished manuscript).

Evolutionary dynamics of sterility mutations

There are two key questions that underlie the 'use it or lose it' hypothesis for the evolutionary degeneration of sex in clonal populations: (1) How do sterile genotypes arise in highly clonal populations? (2) How do sterile genotypes, once they have originated, increase in frequency? There are no general answers to the either of these questions because there has been very little work on the genetics and evolution of sexual infertility. With respect to the first question, it is possible that sterility mutations occur and are maintained at low frequency by clonal propagation in populations where sexual recruitment occurs regularly but is not the predominant mode of recruitment (this probably applies to most clonal plants; Abrahamson, 1980). These mutations may rise in frequency when sexual recruitment becomes rare (Eckert *et al.*, 1999). I am not aware of any study that has quantified the frequency of sterile genotypes in fertile populations of clonal plants.

In populations where sexual recruitment is totally suppressed, sterility due to dominant genetic factors may arise via somatic mutation (Hutchinson, 1975; Klekowski, 1988a). Although somatic mutation is much discussed in the literature, there has been little attempt to determine whether it generates significant genetic variation in clonal populations (Klekowski, 1988c, 1997; Schmid, 1990; McLellan *et al.*, 1997; Eckert, 1999). In sterile polyploids, it is possible that the degeneration of sexual characters is partly due to polyploidy per se
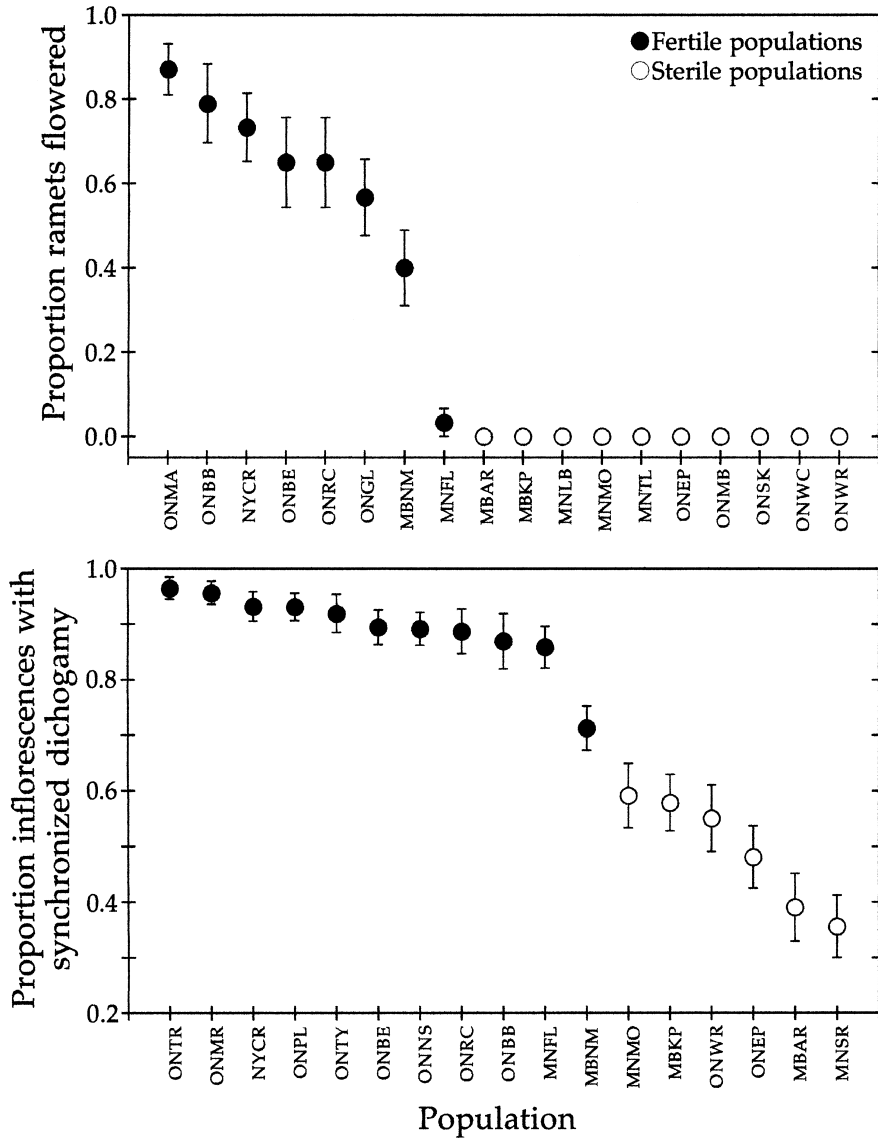
Figure 7. Degeneration of traits related to sexual reproduction in obligately asexual triploid populations of *B. umbellatus* (F.L. Thompson and C.G. Eckert, unpublished data). The top panel shows variation in the proportion of ramets that flowered in a common greenhouse environment based on a sample of about 30 ramets per population. The bottom panel shows variation in the proportion of inflorescences exhibiting synchronized dichogamy in natural populations based on a sample of about 100 inflorescences per population. A similar pattern of variation occurs for the proportion of flowers exhibiting within-flower dichogamy. Error bars are ± 1 binomial SE. Note that triploids also flower at a very low frequency in natural populations. The data on dichogamy for triploids were obtained from large populations of ramets, the vast majority of which were not flowering.

(Levin, 1983) rather than somatic mutations. This could be investigated by comparing sexual characters in synthetic vs. naturally occurring polyploids.

The second question concerning how sterile genotypes increase in frequency is conceptually related to how any type of vestigial character degenerates; a topic which has generated considerable debate (Fong *et al.*, 1995). There are four main hypotheses: (1) neutral mutation; (2) resource reallocation; (3) antagonistic pleiotropy; and (4) mutational meltdown. The neutral mutation hypothesis is the simplest explanation: mutations causing trait degeneration are fixed through a combination of genetic drift and relaxed selection (Brace, 1963; Wilkens, 1988). Although the fixation of individual mutations via drift may be a slow process, complex traits (like sex) governed by many loci might degenerate rapidly because they present a large target to mutation and drift (Culver, 1982). The resource reallocation hypothesis states that the fixation of mutations which reduce a vestigial trait, sterility mutations in this case, would be facilitated if the reduction in the nonfunctional trait freed up resources that could be invested in traits that increase fitness (Poulson and White, 1969; Regal, 1977). For example, reduced flower production (e.g. triploid *B. umbellatus*) may allow increased vegetative growth, clonal reproduction and/or ramet survival. The antagonistic pleiotropy hypothesis states that degenerative mutations may increase in frequency quickly via selection if they have pleiotropic effects on other traits that increase fitness (Prout, 1964; Wright, 1964). Finally, the mutational meltdown hypothesis proposes that sexual dysfunction in highly clonal populations is part and parcel of a decrease in overall vigour due to the accumulation of generally deleterious mutations in the absence of sex (Lynch *et al.*, 1993).

There are almost no data with which to evaluate these alternative hypotheses. The four hypotheses can be distinguished to some extent by determining whether genotypes with reduced sexuality exhibit enhanced performance in terms of survival, vegetative growth or clonal propagation. The neutral mutation hypothesis predicts that there should be no difference in vegetative performance between sterile and fertile genotypes. That is, the mutations that reduce sexual capacity do not increase or decrease components of fitness. In contrast, the resource reallocation and antagonistic pleiotropy hypotheses predict that sterile genotypes should exhibit higher vegetative vigour than fertile genotypes. The mutational meltdown hypothesis predicts that vegetative vigour correlates negatively with sexual sterility.

Studies with *D. verticillatus* failed to reveal any increase or decrease in vegetative vigour associated with sexual sterility, thereby supporting only the neutral mutation hypothesis. In a greenhouse experiment involving F1s from reciprocal crosses within and among infertile and fertile populations, sterile progeny did not exhibit particularly high or low vegetative growth or survival (Eckert *et al.*, 1999). Preliminary results from a larger-scale experiment con-
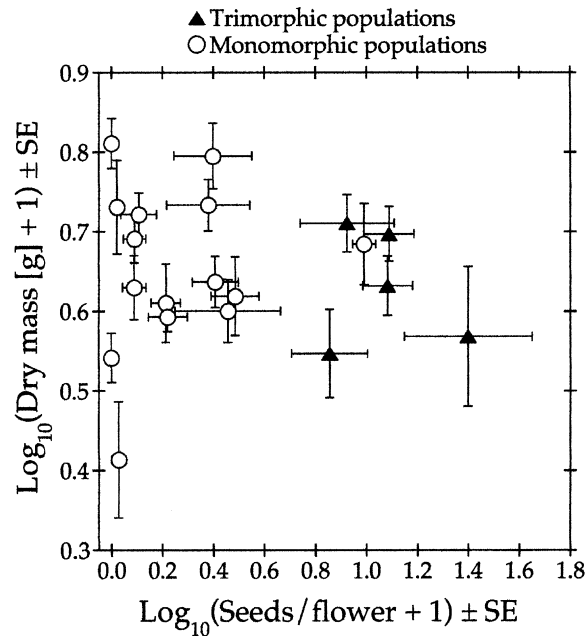
Figure 8. Sexual infertility is not associated with increased or decreased vegetative vigour among populations of *D. verticillatus* (C.G. Eckert, F.L. Thompson and M.E. Dorken, unpublished data). Population means ± SE for dry mass (vegetative vigour) are based on data from three clonal replicates from each of nine ramets per population grown for 6 months in a common greenhouse environment. Seeds/flower (sexual fertility) was measured by hand-pollinating, ramets in a common greenhouse environment (as in Fig. 5). Sexual fertility does not correlate with vegetative vigour (Pearson $r = -0.044$, $n = 20$, $p = 0.85$).

trasting genotypes from 18 infertile and seven fertile populations grown in a common greenhouse environment also failed to reveal any covariation between sexual sterility and vegetative performance (Fig. 8). A more powerful experimental test of these hypotheses would involve creating a variable F2 from crosses between fertile and near-infertile genotypes, planting it into geographically central and marginal populations, and testing for positive covariation between sexual sterility and aspects of vegetative performance. If an advantage of sterility was detected, experimental removal of reproductive meristems could be used to distinguish between the resource reallocation and antagonistic pleiotropy hypotheses. The latter would be supported if vegetative superiority of sterile genotypes persists when all plants are prevented from investing in sex.

Promising directions for future research

Plant ecologists have long recognized that many plants combine sexual and clonal reproduction and that the balance between these reproductive modes

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may vary widely between and within species. Broad variation in reproductive mode is also expected to have significant consequences for the ecology, genetics and evolution of clonal plants. Yet, relatively few studies have investigated the extent of sexual variation among populations of clonal plants species and only a handful have attempted to determine the underlying ecological and/or genetic causes. There is a need for much more work on this general issue, and here are some specific questions that will probably reward serious investigation.

(1) *Does wide variation in sexual reproduction lead to wide variation in genotypic diversity*? Broad comparisons of population genetic structure have revealed that, on average, clonal plants are not that different from predominantly sexual plants. I argue that we should pay more attention to the variance in genotypic diversity than the mean, and seek to identify the mechanisms causing variance.

(2) *What is the relative importance of sexual recruitment, immigration and competitive exclusion in modulating genotypic diversity in clonal plant populations?* Progress on this question is likely to be enhanced by the increased availability of hypervariable genetic markers (e.g. AFLPs and microsatellites) plus new spatially explicit statistical analyses.

(3) *What is the genetic architecture of sexual sterility?* Work on *D. verticillatus* and *S. erectum* suggests that the capacity for sexual reproduction in clonal plants is evolutionarily labile. What are the genetic mechanisms? Because sterility is rarely complete, quantitative genetics, including QTL mapping, (Lynch and Walsh, 1998) could be profitably used to address this question. Understanding the genetics of sexual sterility in model systems like *Arabidopsis* (Preuss, 1995) may also provide the genetic and biochemical details of the mechanisms underlying sexual variation in clonal plants.

(4) *How does sexual sterility evolve in clonal plants?* Is the genetic loss of sexual fertility associated with enhanced vegetative growth, survival and/or clonal propagation? To what extent might this association be due to resource vs. genetic trade-offs?

(5) *What are the evolutionary consequences of wide sexual variation in clonal plants?* Although there has been some discussion of how clonal structure affects the operation of natural selection on ramets vs. genets (Vuorisalo *et al.*, 1997), there has been little consideration of larger-scale evolutionary consequences. For instance, how might sexual variation affect local adaptation and the evolution of the geographical range. Recent theoretical work suggests that adaptation at the range limit or in ecologically marginal habitats is hampered by recurrent gene flow from larger, more productive, central populations (Kirkpatrick and Barton, 1997; Case and Taper, 2000). To what extent do these models apply to plants, many of which are clonal? Work on *D. verticillatus* suggests that populations at the northern range margins are sexually sterile, possess very limited genotypic diversity, and probably experience little gene flow (Dorken and Eckert, 2001). Is this by itself sufficient to limit the

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geographical range? To what extent does predominant clonal recruitment in marginal populations reduce the immigration of genes from central populations? How might reduced population genetic diversity and dispersal alter metapopulation dynamics at the range margin (Holt and Keitt, 2000)? If severe reductions in sexual recruitment near the range limit are common in clonal plants, the theoretical framework for understanding the evolution of range limits may require broadening to address these questions.

Acknowledgements

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