THE EVOLUTIONARY MAINTENANCE OF SEXUAL REPRODUCTION: EVIDENCE FROM THE ECOLOGICAL DISTRIBUTION OF ASEXUAL REPRODUCTION IN CLONAL PLANTS

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In theory, females that reproduce asexually should enjoy a twofold advantage in fitness over sexual females, yet sex remains the predominant mode of reproduction in virtually all eukaryotes. The evolutionary maintenance of sex is especially puzzling in clonal plants because the transition from sexual to exclusively asexual reproduction is an ever-present possibility in these species. In this article, I use published data on the genotypic diversity of populations of clonal plants to test five hypotheses about the ecological situations that limit or favor clonal reproduction in vascular plants. The data were drawn from 248 studies covering 69,000 individuals in >2000 populations of 218 species in 74 plant families. The tests showed the following: (1) the frequency of clonality increases with population age, indicating that clonal reproduction is limited by disturbance; (2) clonal reproduction is limited by dispersal; clones are more frequent in aquatic and apomictic species in which the dispersal of clonally produced propagules is less limiting; (3) clones are more frequent in populations of rare or endangered species; (4) populations of alien plants have higher frequencies of clonality; and (5) clones are more frequent at the edges of species' geographical ranges. Thus, it appears that the ultimately successful clonal plant would be a rare, aquatic, alien apomict living in an undisturbed, geographically marginal habitat. Since this combination of circumstances is so restrictive, it is perhaps better regarded as a sign of sexual failure than as a recipe for clonal success.

Keywords: apomixis, aquatic plants, clonal plants, dispersal, evolution of sex, genotypic diversity.

Introduction

The maintenance of sexual reproduction remains an evolutionary problem for which there are many contending solutions (Williams 1975; Kondrashov 1993; Otto and Lenormand 2002; Rice 2002). The puzzle is why females should continue sexual reproduction when this dilutes their genetic contribution to the next generation by half compared to asexual reproduction (Maynard Smith 1978). The problem is similar for outcrossing hermaphrodites, a category that includes the majority of seed plants. In fact, plants present the problem in its most acute form because sexual reproduction persists even though so many plants are clonal and capable of asexual reproduction (Lloyd 1980). Somatic embryogenesis is phylogenetically ancient in the plant kingdom (Mogie and Hutchings 1990), but only very rarely do clonal plants become entirely asexual (Eckert 2001; Pandit and Babu 2003). Why is the transition to complete asexuality so rare, even in organisms so well equipped to reproduce clonally?

The combination of sexual and asexual reproduction within plant life histories presents an opportunity as well as a challenge. The opportunity is to compare the relative success of recruitment through sexual and asexual routes across a broad range of clonal species in order to test under what conditions one mode of reproduction is favored over the other. It would be possible, through parentage analysis (Smouse et al. 1999; Burczyk et al. 2006), to measure the relative success of sexual

versus asexual reproduction directly, though at the time of writing, parentage analysis had not been used for this purpose. As an alternative, the genotypic diversity of a population can be used as an indirect measure of reproductive success via sexual and asexual routes (see "Methods").

Asexual reproduction in plants occurs in two fundamentally different forms: vegetative reproduction and agamospermy. In clonal plants, vegetative reproduction produces new ramets by budding from roots, rhizomes, stems, storage organs such as tubers or (more rarely) leaves, or inflorescences. The earlier literature on clonal plants tended to emphasize the ecological role of vegetatively produced organs in the growth of individual plants and populations and to neglect the evolutionary implications that arise from the fact that the same organs effect asexual reproduction (Eckert 1999).

The vegetative progeny, such as bulbils, of clonal plants tend to be better provisioned than seeds, or they start life as miniature versions of their mothers, complete with their own root systems. How long the vascular connection between mother and daughter endures varies within (Alpert 1999) and between species (Van Groenendael et al. 1997). Obligate vegetative reproduction, without even occasional sexual reproduction, may be selected against (and hence be rare) because of the mutational load that may accumulate (Kondrashov 1994; Caetano Anolles 1999; Paland and Lynch 2006), although mechanisms exist to purge mutations within individual plants (Thomas 2002), and these lower mutational load (Orive 2001).

Agamospermy is parthenogenetic seed production, also referred to as apomixis (in the narrow sense; Askers and Jerling 1992). Apomictic seeds are clones of the mother plant, but

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they are packaged and dispersed in the trappings of sexually produced progeny. Because apomictic seeds share the same dispersal mechanisms and require the same conditions for establishment as sexually produced seeds, they can be used to test the performance of clones without the normal dispersal handicap of vegetative reproduction (Mogie et al. 1990). Some apomictic (pseudogamous) species require pollination as a trigger for seed development, even though pollen makes no genetic contribution to the seed (Richards 2003). Stable coexistence of apomictic and sexual seed production within the same population appears to be difficult to achieve, and mixtures of the two reproductive modes are thought not to occur (Bengtsson and Ceplitis 2000; Nakayama et al. 2002; Whitton et al. 2008), although apomicts may very occasionally produce seeds by outcrossing (Richards 2003; Thompson et al. 2008). Apomixis is much rarer than vegetative reproduction among flowering plants (Whitton et al. 2008) and is often accompanied by polyploidization (Bierzychudek 1987; Richards 2003). One possible reason for this is that polyploidy protects apomicts from the expression of recessive mutations that accumulate in asexual genomes (Archetti 2004). A full discussion of apomixis is given by Whitton et al. (2008).

Successive reviews of genetic variation in clonal plants (Ellstrand and Roose 1987; Widén et al. 1994; Diggle et al. 1998; Khudamrongsawat et al. 2004) have all amply demonstrated that such populations are not genetically depauperate. Theoretical studies confirm that genetic diversity, as measured by alleles per locus or heterozygosity, is not expected to decrease and may even increase with increasing asexuality if clones are heterozygous (Balloux et al. 2003). However, both models and data show that the diversity of genotypes is reduced by asexual reproduction and that some populations may become monomorphic.

Models that examine the conditions under which sexual and asexual reproduction can coexist have found that sexual reproduction will persist if there is temporal variation in resource supply that clones cannot track (Weeks 1993). A life-history strategy combining sexual and asexual reproduction can be stable if the environment varies and the two modes of reproduction are successful in different circumstances (Bengtsson and Ceplitis 2000). Both these models emphasize the inflexibility of clonal reproduction when the environment varies. The view that clonally reproducing organisms are evolutionarily handicapped by a lack of genetic variation has been challenged on the grounds that clones often harbor hidden variation that enables them to adapt (Lushai et al. 2003). If this is true for clonal plants, then the persistence of sexual reproduction is all the more a puzzle.

The converse of the argument that environmental change favors sexual reproduction is that a lack of change should favor clonality. Two types of environmental change should be distinguished: episodic change, such as that caused by habitat disturbances such as fire or tree falls, and continuous change, such as climatic warming. Following Bender et al. (1984), I use the terms "pulse" and "press," respectively, for these two types of perturbation. Pulse perturbations occur in all kinds of plant communities and provide essential recruitment opportunities for many species that usually colonize from seed. As time since the last disturbance elapses, opportunities for further colonization from seed diminish, and the size of the most

successful clonal genotypes that have already established increases. Competition between clones is expected to lead to a fall in genotypic diversity over time (Gray 1987). The rate of clonal spread during this phase is an important contribution to the fitness of the genet (Pan and Price 2001). Press perturbation is no doubt as common as pulse perturbation, but it is much more difficult to test its effect on the relative success of sexual versus clonal reproduction in perennial plants because controls for gradual environmental change are rarely available over a sufficient period.

The long- and short-term advantages of sexual reproduction and, therefore, the corresponding disadvantages of asexual reproduction, have been reviewed elsewhere (Maynard Smith 1978; Bell 1981; Rice 2002; Otto and Gerstein 2006). I confine myself here to the possible limitations and advantages of clonal reproduction that lend themselves to testing by the use of field data.

Clones may be handicapped with respect to sexually produced offspring as follows:

1. Clones are prone to habitat disturbance. Test: sex should be favored over clonal reproduction in disturbed environments, and clones should increase in frequency with the time elapsed since the last disturbance.
2. Clones are poor dispersers. Test: (a) Vegetatively spread clones should be more successful in aquatic habitats because vegetative organs are more easily transported in water than on land (Barrett et al. 1993; Grace 1995); (b) apomicts produce genetically identical (=clonal) seeds that are not handicapped in dispersal with respect to sexually produced seeds. Apomictic populations, therefore, should have higher ratios of clonal/sexual individuals than clonal plants that reproduce vegetatively.

Conversely, clones may be at an advantage with respect to sexually produced offspring in the following situations:

3. In small populations, where sexual fecundity may be lowered by intraclone incompatibility (Nuortila et al. 2002; Honnay and Bossuyt 2005). Test: clones should be more frequent in rare/endangered populations than in common ones.
4. In alien environments, where sexual reproduction may require multiple colonization (Baker 1959). Test: clones should be more frequent in populations of aliens than in populations of natives.
5. At the edge of geographical ranges, where sexual reproduction is subject to physiological limitations. Test: clones should be more frequent in populations at the edge of geographical ranges.

In this article, I analyze the frequency of clonal reproduction in populations of more than 200 plant species to test these five predictions.

Methods

Study Selection

The aim was to include all studies that reported a value for the ratio genotypes per individual sampled (G/N) for one or more clonal plant populations that have been published since

the first review of this subject was conducted 20 years ago (Ellstrand and Roose 1987). I used the ISI Citation Index to identify more than 400 articles that cited Ellstrand and Roose (1987) and then examined each of these studies to identify those that contained suitable data. A small number of relevant studies that did not cite (Ellstrand and Roose 1987) were found in the reference lists of the articles that did do so and among articles that cited them. This search was stopped when it ceased to yield any more unknown studies that had been published before January 1, 2006. I omitted the studies of 21 species used in Ellstrand and Roose's (1987) survey so that the results of my review would be independent of theirs.

Previous surveys of genotypic diversity (Ellstrand and Roose 1987; Widén et al. 1994; Diggle et al. 1998; Hangelbroek et al. 2002) were limited in the number of species covered or confined themselves to studies of populations "in which sexual recruitment is extremely limited" (Ellstrand and Roose 1987, p. 123). I did not apply this restriction because of the difficulty of knowing what it meant in any particular case and because we now know that clonal plants recruit episodically (Eriksson 1989) and that this can easily be missed.

The literature review identified 356 potentially informative studies of population genetic structure in clonal plants, 108 of which had to be rejected because they provided insufficient information. The most common deficiency was the absence of data on genotype frequencies (as distinct from gene frequencies). The 248 qualifying studies between them sampled more than 69,000 individuals in more than 2000 populations representing 218 species in 74 plant families. Some of the larger families, such as Poaceae (29 studies), Asteraceae (18 studies), Cyperaceae (17 studies), and Rosaceae (16 studies), were strongly represented, but relative to their size, so too were smaller families of aquatic plants, such as Alismataceae (six studies), Potamogetonaceae (six studies), Lemnaceae (five studies), Zosteraceae (four studies), and Posidoniaceae (four studies).

More than half of the studies used allozyme markers (58%; 146 studies), 54 studies (22%) used RAPDs, 23 (9%) used nuclear microsatellites, and 17 (7%) used amplified fragment length polymorphisms (AFLPs). Other types of markers used were intersimple sequence repeat (ISSR), RFLP, and chloroplast microsatellite. Nearly half (48%) of the studies were of terrestrial herbs, 17% were of aquatic herbs, 17% were of shrubs, and 12% were of trees. Regarding mode of asexual reproduction, 102 cases were rhizomatous, 30 suckered, 20 were stoloniferous, and 19 were apomictic. Other modes of vegetative reproduction included layering, bulbils, bulbs, tubers, turions, lignotubers, and fragmentation.

Indexes of Clonality and Independent Variables

Four indexes of clonality were used:

1. The ratio G/N of genotypes (G) identified in a population to the number of individuals sampled (N), which (Ellstrand and Roose 1987) called the "proportion distinguishable."
2. An index of genotypic identity (I_G), suggested by Weeks (1993) and closely related to Simpson's index of genotypic diversity.
3. The frequency of monoclonal populations belonging to a species (m).
4. A simple binary variable of presence/absence of clonal populations belonging to a species (M).

It is important to note what these indexes are and what they are not. They are population-level measurements of the net outcome of all processes that in the history of a population affected its genotypic diversity, including vegetative reproduction, sexual reproduction, and immigration by sexually and asexually produced propagules. The indexes are not direct estimates of the fitness of plants that reproduce sexually versus the fitness of those that reproduce vegetatively. However, I do assume that the indexes give an indirect estimate of the likely reproductive success of plants via sexual and asexual routes.

The indexes were correlated with one another (see "Results"), and none of them is unbiased, but all have the practical advantage that they are reported in or can be calculated from many studies. The index G/N reflects the ratio of recruitment into the population via sexual versus asexual reproduction if the population is at demographic equilibrium and if all identical genotypes are in fact clonemates. There are two reasons why the latter condition could be incorrect. First, if too few polymorphic loci have been used in clone discrimination, some apparent clonemates may have undetected differences at loci that were not sampled. The presence of this problem can be diagnosed by increasing the number of loci until the number of clones detected asymptotes. Second, some proportion of apparent clonemates may actually be the product of sexual reproduction. This problem is less easily solved, though there are several possible solutions based on allele frequencies and intersample distances (Parks and Werth 1993; Harada et al. 1997; Stenberg et al. 2003; Meirmans and Van Tienderen 2004; Halkett et al. 2005). Some recent studies of genotypic diversity in clonal plants have used these clone-discrimination techniques (e.g., Ivey and Richards 2001; Douhovnikoff and Dodd 2003), but the majority have not. In most cases, the data required to apply clone discrimination techniques retrospectively are not published. Incorrect clone discrimination is less likely to be a problem as the number of marker loci increases. A source of bias that could inflate G/N and other estimates of clonal diversity is somatic mutation (Klekowski 2003). This is particularly likely to inflate the number of genotypes scored using DNA markers such as microsatellites that are sensitive to sequence differences of a single base pair between samples. The influence of somatic mutation on genotype diversity has been considered, though rarely quantified, by a number of studies (Tuskan et al. 1996; Esselman et al. 1999; Torimaru et al. 2003; Van der Hulst et al. 2003; Nagamitsu et al. 2004; Kameyama and Ohara 2006; Kjolner et al. 2006). Somatic mutation is often undetectable (Kameyama and Ohara 2006) or occurs only at a very low rate (Cloutier et al. 2003).

The index of genotypic identity (I_G) is the probability that any two individuals drawn at random from a population have the same genotype (Bengtsson 2003). In entirely clonal populations, this will approach unity, while in entirely sexual populations, it will tend to zero, so I_G can be used as an index of clonality for a population. If a population has been at demographic equilibrium for sufficient time, I_G approaches a value
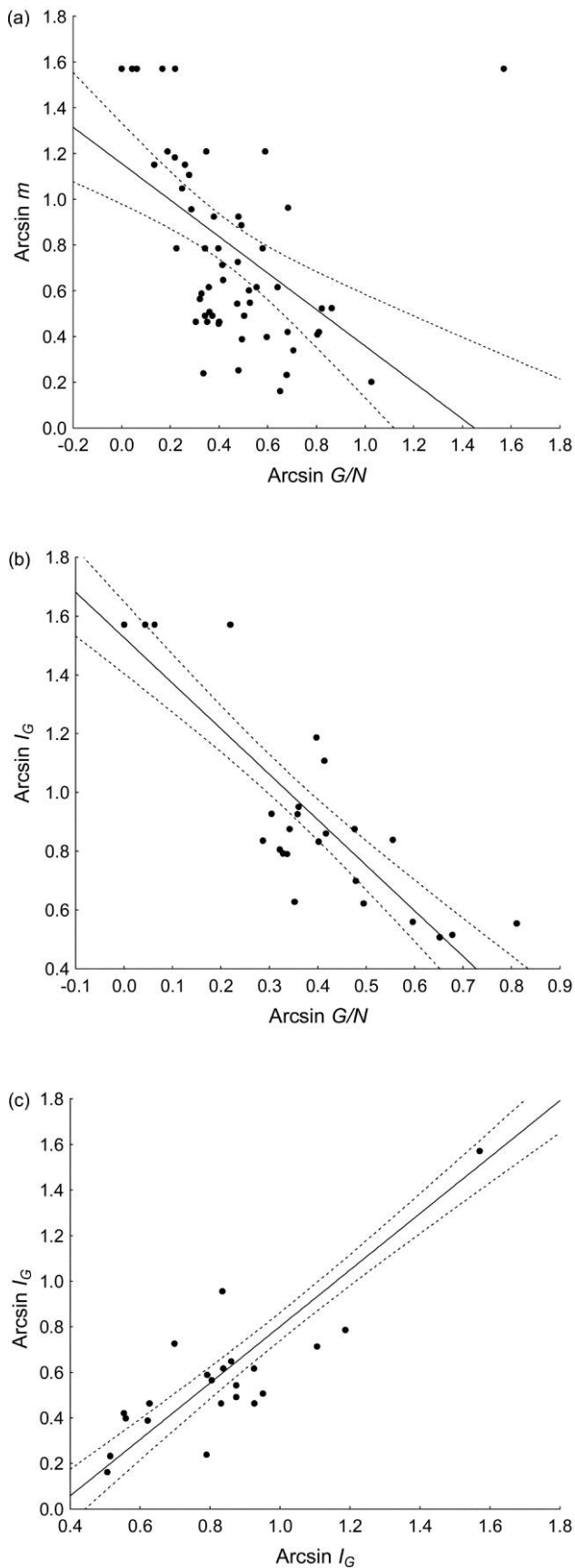
independent of starting conditions. When the proportion of individuals derived from sexual reproduction, σ, is small ($\sigma < 0.10$) and the population size is sufficiently large ($N > 10$), genotypic identity can be approximated (Bengtsson 2003) as

$$I_G \approx \frac{1}{2\sigma N + 1}.$$

Following the example of Ellstrand and Roose (1987) and the recommendation of Parker (1979), virtually all studies of genotypic diversity included in this survey calculated Simpson's index of diversity (D), corrected for finite sample size. Conveniently, like I_G, this also gives the probability that any two individuals drawn at random from a population have the same genotype:

$$D = \sum \frac{n_i(n_i - 1)}{N(N - 1)},$$

where n_i is the number of individuals belonging to the ith genotype and N is the total number of individuals. Simpson's index is invariably reported as the complement of D, so it increases from 0 to 1 as diversity increases. The complement of reported values of Simpson's index was therefore used to estimate I_G.

Statistical Analysis

Statistical models of each of the four indexes of clonality were run with a common set of independent variables. These variables were growth form (terrestrial herb, aquatic herb, shrub, tree, vine, succulent), rarity (scored "rare" if the source described the species as rare, endangered, or narrow endemic and scored "not rare" in all other cases), alien status (alien vs. native), and type of clonality (apomictic vs. vegetative). Interactions among independent variables were not examined.

Four covariates were also used to control for sampling bias. Since DNA markers tend to yield more polymorphic loci than allozymes, and anonymous DNA markers (RAPDs, ISSRs, AFLPs) are prone to artefactual variation caused by sample contamination and PCR error, use of the different markers could clearly influence the number of genotypes detected in a population. Possible bias from the use of different types of genetic markers was controlled for by coding whether studies used DNA markers or allozymes. Bias also could have arisen because some studies sampled populations using a spacing interval between samples designed to reduce the probability of sampling the same clone more than once. These studies could have been omitted altogether, but since the data showed that the same clones were in fact often resampled, I included the studies and flagged them with a binary covariate instead. Two other possible sources of bias were included as covariates in initial models: number of populations sampled for a species and an index of the spatial scale of sampling used.

Species that were the subject of more than one independent study were combined so that no species was represented more

Fig. 1 Correlations among the three continuously distributed indexes of clonality. Dashed lines are 95% confidence limits for linear regression lines. *a*, Arcsin $m = 1.1564 - .7977 \times$ arcsin genotype/sample ratio (G/N), $r = -.5074$; *b*, arcsin $I_G = 1.5264 - 1.548 \times$ arcsin (G/N), $r = -.8940$; *c*, arcsin $m = -.4384 + 1.2391 \times$ arcsin I_G, $r = 0.9494$.

than once in the data set. The sole exception where studies were not combined was the aquatic species *Butomus umbellatus*, because one study examined native European populations (Kirschner et al. 2004) and the other one studied alien North American populations (Eckert et al. 2003).

A combined value of G/N was calculated from individual study values of G_i and N_i as $\Sigma G_i/\Sigma N_i$. A species value of D was calculated as the arithmetic mean of D_i for individual studies. The percentage of polymorphic populations for a species was calculated as $(\Sigma p_i/\Sigma s_i) \times 100$, where p_i was the number of polymorphic populations and s_i was the total number of populations in an individual study.

All statistical tests were performed using STATISTICA, release 7 (StatSoft 2000). Separate general linear models of the arcsin-transformed values of G/N, I_G, and m were run. All independent variables and covariates were included in initial models, which were then repeated with nonsignificant variables dropped sequentially to obtain an optimum model. A generalized linear model with a binomial distribution and a logit link function for presence/absence of monoclonal populations (M) was run using the same sequential procedure. In tests of the disturbance and latitude hypotheses, the index G/N was compared between young and old populations and between southern and northern populations, respectively, across all relevant studies and using Wilcoxon matched-pairs tests (StatSoft 2000).

Results

Correlation among Clonality Indexes

As expected, the indexes of clonality were correlated with each other, but the relationships among the three continuously distributed variables (G/N, I_G, m) showed that they were not equivalent (fig. 1). Arcsin I_G was highly correlated with arcsin G/N ($r = -0.894$, $n = 29$, $P < 0.001$) and m ($r = 0.949$, $n = 29$, $P < 0.001$), but the sample size was small, so this variable is of limited use within the current data set. Arcsin G/N and arcsin m were not highly correlated with each other even though the relationship was significant ($r = -0.507$, $n = 61$, $P < 0.001$; fig. 1).

Change in G/N with Population Age

Twenty-two studies included populations with different times since last disturbance or populations of different successional ages. Two studies, both of coastal grasses (Bockelmann et al. 2003; Travis and Hester 2005), included sufficient populations of different age for regression to be used to test how G/N changed with time. In *Elymus athericus* (Bockelmann et al. 2003), regression of G/N on age for nine populations for which an age was given by the authors showed no significant change over time ($G/N = 0.0003$ age $+ 0.8777$, $r^2 = 0.067$). The results of Travis and Hester (2005) for *Spartina alterniflora* are mentioned in the "Discussion." The remaining 20 studies analyzed fewer than five population types (usually only two). A significant decline in G/N occurred with population age in the sample of 20 studies between the youngest ($\overline{X} = 0.604$, SE $= 0.058$) and oldest populations ($\overline{X} = 0.437$,

SE $= 0.068$; Wilcoxon matched-pairs test: $n = 20$, $Z = 2.24$, $P = 0.025$). Two of the three strong exceptions to this trend (fig. 2) were seagrasses.

Monoclonal Populations

In generalized linear models of M in 169 species, growth form ($\chi^2 = 11.567$, df $= 3$, $P = 0.009$), rarity ($\chi^2 = 10.55$, df $= 1$, $P = 0.001$), and the number of populations ($\chi^2 = 20.59$, df $= 1$, $P < 0.0001$) were all highly significant. In a comparison of growth forms, the majority of aquatic herbs had monoclonal populations, while the majority of species with other growth forms did not (fig. 3). A general linear model of arcsin m (the proportion of populations that were monoclonal) also showed that rarity had a highly significant effect ($F_{1,163} = 11.700$, $P < 0.001$), in addition to growth form ($F_{3,163} = 4.806$, $P = 0.003$) and number of populations ($F_{1,163} = 7.876$, $P = 0.006$; fig. 4). The mean value of m computed from the model was 22.46% (SD $= 0.84\%$) for the rare species in the sample, compared with mean m of only 5.57% (SD $= 0.14\%$) in populations of species that were not rare. The sample of 169 species excluded eight species of succulents and vines because of the small sample size for these two growth forms, but analyses that included these species gave almost identical results.
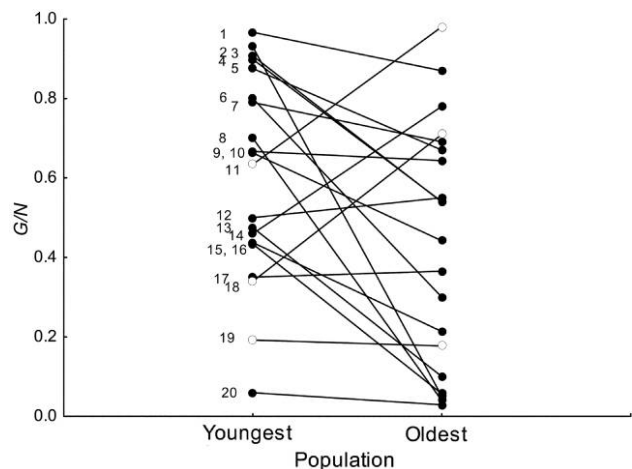

Fig. 2 Ratio of genotypes/sample (G/N) compared between young and old populations drawn from 20 studies of 19 clonal species. Seagrasses are indicated by open circles. Key to species: 1 = *Populus nigra* (Barsoum et al. 2004), 2 = *Calamagrostis epigejos* (Lehmann 1997), 3 = *Veratrum album* (Kleijn and Steinger 2002), 4 = *Oryza rufipogon* (Xie et al. 2001), 5 = *Empetrum hermaphroditum* (Szmidt et al. 2002), 6 = *Agrostis stolonifera* (Kik et al. 1990), 7 = *Cirsium arvense* (Sole et al. 2004), 8 = *Solidago altissima* (Maddox et al. 1989), 9 = *Psammochloa villosa* (Li and Ge 2001), 10 = *Andropogon gerardii* (Keeler et al. 2002), 11 = *Zostera marina* (Reusch et al. 2000), 12 = *Brachypodium pinnatum* (Schlapfer and Fischer 1998), 13 = *Rhododendron ferrugineum* (Pornon et al. 2000), 14 = *Circaea lutetiana* (Verburg et al. 2000), 15 = *Carex lasiocarpa* (McClintock and Waterway 1993), 16 = *Festuca rubra* (Rhebergen et al. 1988), 17 = *Carex pellita* (McClintock and Waterway 1993), 18 = *Posidonia oceanica* (Jover et al. 2003), 19 = *Zostera marina* (Rhode and Duffy 2004), and 20 = *Uvularia perfoliata* (Kudoh et al. 1999).
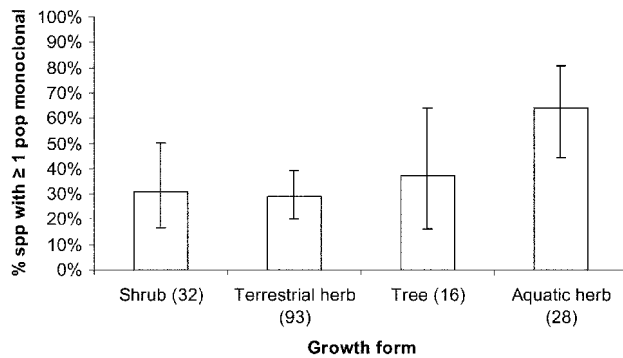
Fig. 3 Percentage of species in each of four growth forms that possessed at least one monoclonal population; 95% binomial confidence limits corrected for continuity are shown. Numerals in parentheses are sample sizes (n).

Ecological Correlates of G/N

A sample of 200 species was analyzed; vines and succulents were excluded because few of these plants were represented. In the final general linear model of the arcsin-transformed ratio G/N, growth form ($F_{3, 193} = 5.066$, $P = 0.002$), type of clonality ($F_{1, 193} = 7.424$, $P = 0.007$), alien status ($F_{1, 193} = 10.318$, $P = 0.002$), and marker type ($F_{1, 193} = 24.597$, $P < 0.0001$) were all significant (fig. 5).

Ecological Correlates of I_G

Values of I_G were available for 106 species in the data set. In the final general linear model of arcsin-transformed values of I_G, just two variables were significant: type of clonality ($F_{1, 103} = 5.449$, $P = 0.0215$) and rarity ($F_{1, 103} = 7.1332$, $P = 0.009$) (fig. 6).

Effect of Latitude on G/N

A barely significant decline in G/N occurred with increasing latitude. In the sample of 17 studies, southern populations had a higher mean value of G/N ($\overline{X} = 0.502$, SE $= 0.055$) than northern ones ($\overline{X} = 0.402$, SE $= 0.069$; Wilcoxon matched-pairs test: $n = 17$, $Z = 1.965$, $P = 0.0495$; fig. 7).

Discussion

Twenty years ago, Ellstrand and Roose (1987, p. 127) concluded, "The striking fact from the data we compiled is that the vast majority of species studied are multiclonal." A later review of a larger sample of 45 species (Widén et al. 1994) reached much the same conclusion. Others have questioned these conclusions on the grounds that the samples were subject to a variety of sources of bias (Eckert 2001; Honnay and Bossuyt 2005). In this study, I controlled for bias by using marker type (DNA markers vs. allozymes), scale of sampling, sampling design, and number of populations sampled as covariates and by analyzing a data set that was nearly five times larger than any used before. The results showed that Ellstrand and Roose's "striking fact" holds true as a broad generaliza-

tion, but it must be qualified by exceptions that form some clear ecological patterns.

In the current data set, clones were more prevalent in older, longer-established populations than in younger populations of the same species (fig. 2), indicating that the absence of disturbance favors asexual over sexual recruitment. The exceptions to this trend are informative. There were only three seagrasses in the sample, but in two of them, the ratio G/N increased rather than decreased with age. Vegetative parts of aquatic plants are much more easily dispersed than are clonal fragments of terrestrial plants, so the pattern that occurs on land, where populations tend to be established by sexual propagules and expand by asexual reproduction, may be reversed in water. Two studies of chronosequences of coastal grasses were not included in my sample. These plants are not aquatic, but their populations are regularly inundated, and fragments can be dispersed by water. In one study, *Spartina alterniflora* (Travis and Hester 2005) G/N declined very slowly with population age over a period of 1500 yr, while in the other, *Elymus athericus* (Bockelmann et al. 2003) G/N did not change significantly (see "Results"). In no case did the trend toward lower G/N with age terminate in a monoclonal population, indicating that very long timescales free from disturbance may be needed to allow clonal reproduction to prevail over sexual reproduction.
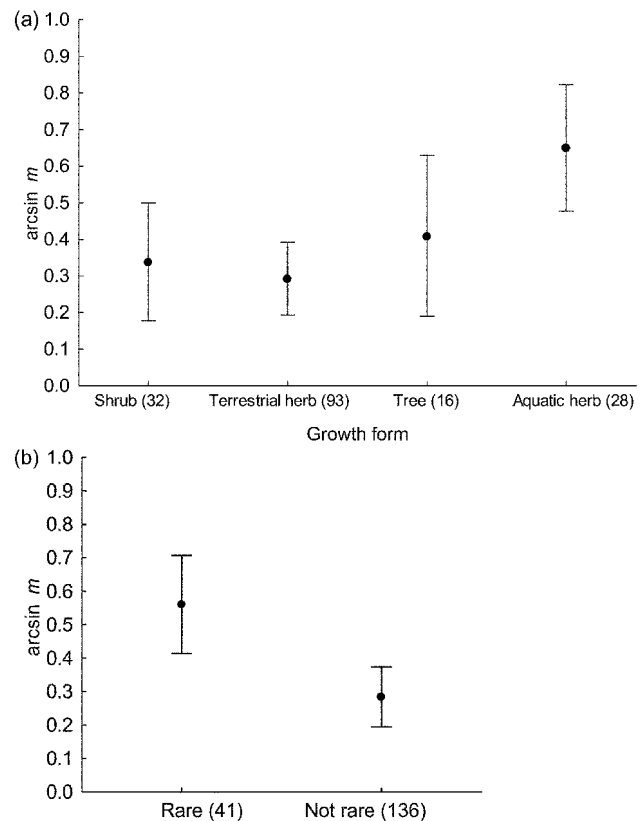

Fig. 4 Means and 95% confidence intervals of arcsin m for species with different growth forms (*a*) and rare versus other status (*b*). Numerals in parentheses are sample sizes (n).
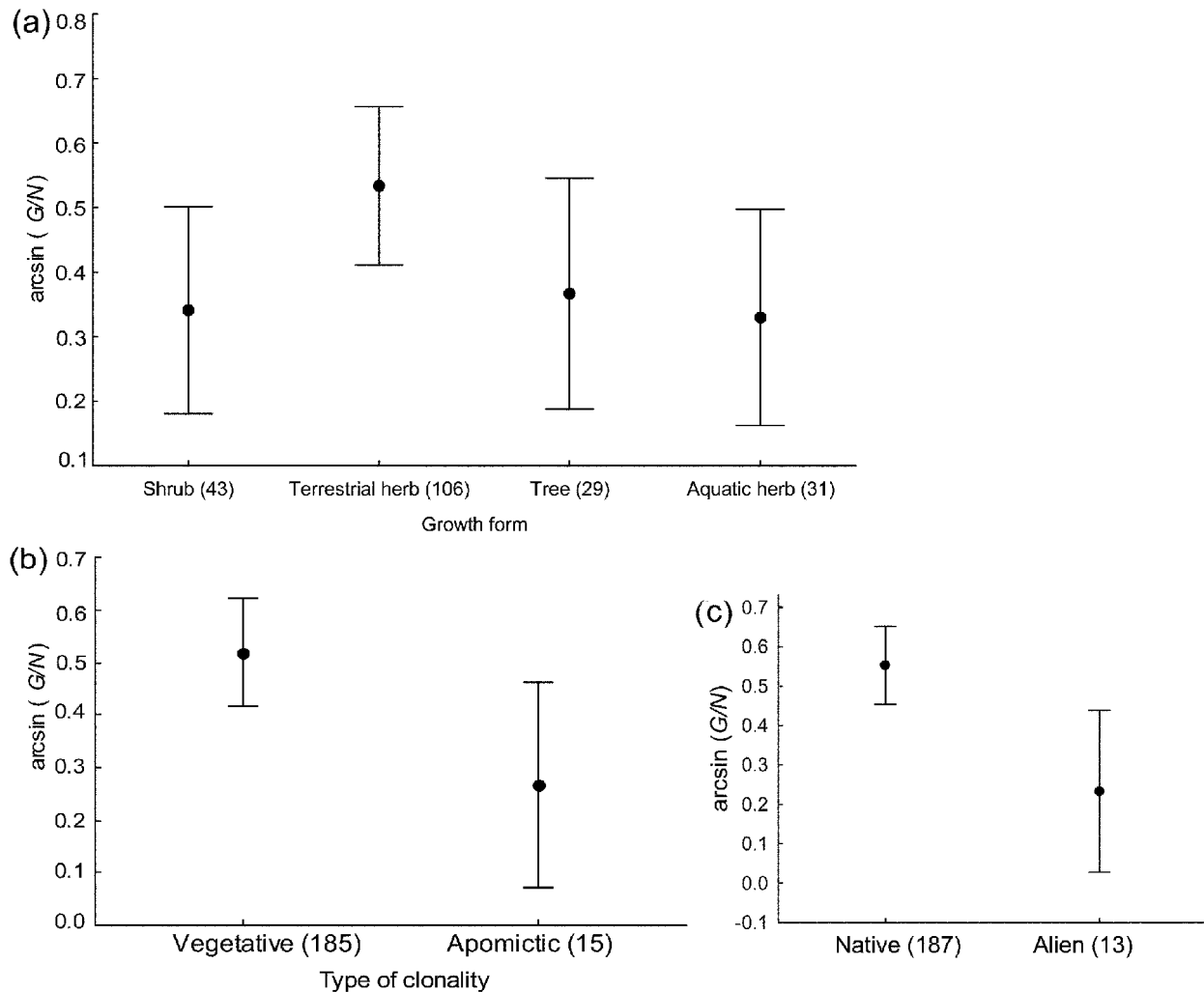
Fig. 5 Means and 95% confidence intervals of arcsin (genotype/sample ratio G/N) for species with different growth forms (*a*), vegetative versus apomictic asexual reproduction (*b*), and native versus alien status (*c*). Numerals in parentheses are sample sizes (*n*).

It follows from the greater dispersability of plant fragments in water than on land that aquatic plant populations should be more clonal, because new populations can more easily be founded by single genotypes. This prediction was strongly supported by results for the clonality indexes M and m. The majority of aquatic herbs in which more than one population was studied contained at least one population that was monoclonal (fig. 3). By contrast, in terrestrial herbs, shrubs, and trees, the majority of species contained no monoclonal populations. Aquatic herbs also had a higher proportion of monoclonal populations (m) than other growth forms (fig. 4). These trends were not apparent when the other two clonality indexes were used, G/N and I_G. The sample of aquatic herbs for which I_G was known was small ($n = 13$), so the test had low power and no conclusion can be drawn for this index, but this was not the case for the G/N sample ($n = 31$). In the study as a whole, the indexes m and G/N were not highly correlated (fig. 1*a*), suggesting that they measure different processes.

The results of the test of the disturbance hypothesis might explain why the monoclonality indexes (m, M) and G/N give different results for aquatic herbs. I suggested that seagrasses were exceptions to the decline in G/N with time because aquatic populations frequently establish from single vegetative fragments and because they acquire rather than lose clonal diversity with time. If the rate at which G/N rises over time in aquatic plants is faster than the rate at which G/N declines over time in terrestrial species, it is possible for aquatic and terrestrial species to have similar average values of G/N, even though they differ significantly in values of M and m. In short, aquatic populations are easily and frequently founded by single clones and move rapidly away from monoclonality, while terrestrial species move very slowly toward it from a sexual beginning.

Because apomicts can disperse their clonal progeny in seeds, thus avoiding the handicap that affects vegetative clonal offspring on land, they too should have higher frequencies of clonality. This was borne out by G/N (fig. 5*b*) and I_G (fig. 6*a*), but not by M or m. It would appear from this that apomicts behave like other seed-producing terrestrial plants as regards the frequency of monoclonal populations but that apomictic
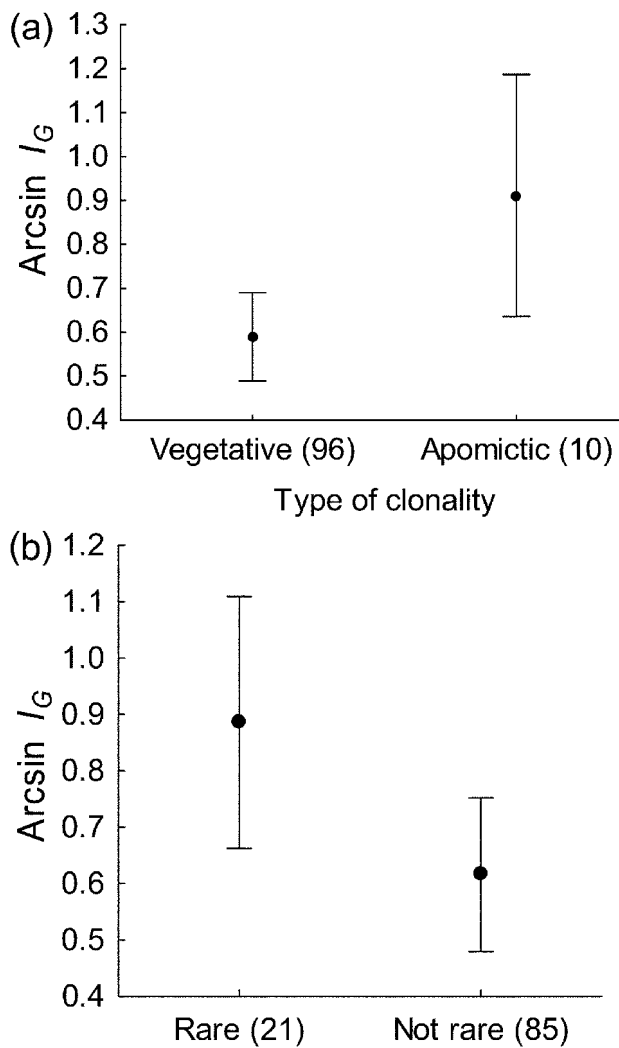
Fig. 6 Means and 95% confidence intervals of arcsin I_G for species with vegetative versus apomictic asexual reproduction (*a*) and rare versus other status (*b*). Numerals in parentheses are sample sizes (*n*).

latitude effect shown in figure 7 (no. 16). In nonclonal animals and plants, threatened species often have lower genetic diversity than related nonthreatened taxa (Eckert 2001; Klekowski 2003; Spielman et al. 2004).

Failure of sexual reproduction is common in small plant populations (Leimu et al. 2006). Is low genotypic diversity merely a passive by-product of sexual failure, or is there active selection in favor of clonal reproduction in such populations? The evidence on this point is slim, but one study found evidence for a trade-off between sexual function and vegetative growth in the clonal aquatic plant *Decodon verticillatus* (Dorken et al. 2004). Sexual reproduction has repeatedly been lost in marginal populations of *D. verticillatus*, presumably because this enhances clonal growth and increases fitness. I am not aware of any other studies that have demonstrated this trade-off in a plant where sexual reproduction has been lost in small or marginal populations. However, the trade-off has been found in other clonal plants (Bullock et al. 1995; Sutherland and Vickery 1988; van Kleunen et al. 2002) and may be general, in which case selection for loss of sexual reproduction would not be confined to the one example that is known. However, if a trade-off between
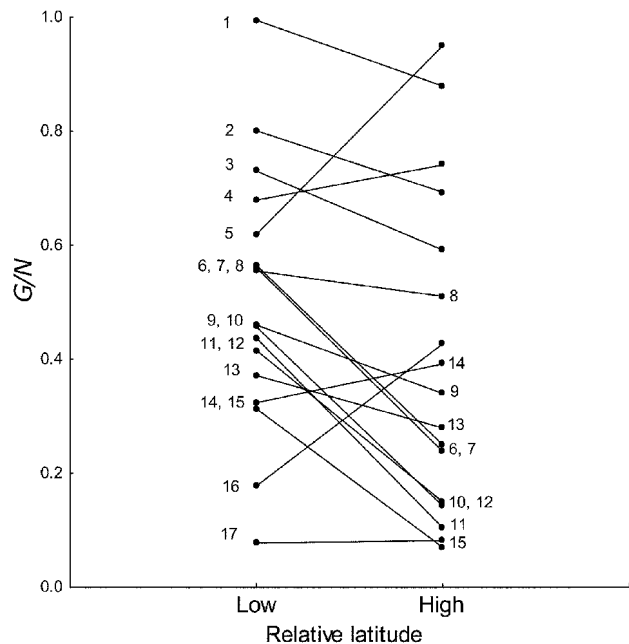

Fig. 7 Ratios of genotypes/sample size (*G/N*) compared between southern and northern populations. (Latitudes have been reversed for the Southern Hemisphere species 6 and 8). Species and sources of data are: 1 = *Corylus avellana* (Persson et al. 2004), 2 = *Zostera marina* (Reusch et al. 2000), 3 = *Cirsium heterophyllum* (Jump et al. 2003), 4 = *Titanotrichum oldhamii* (Wang et al. 2004), 5 = *Carex lugens* (Stenstrom et al. 2001), 6 = *Posidonia oceanica* (Jover et al. 2003), 7 = *Carex sifolia* (Stenstrom et al. 2001), 8 = *Eucalyptus curtisii* (Smith et al. 2003), 9 = *Cirsium arvense* (Jump et al. 2003), 10 = *Carex bigelowii* (Stenstrom et al. 2001), 11 = *Decodon verticillatus* (Dorken and Eckert 2001), 12 = *Carex stans* (Stenstrom et al. 2001), 13 = *Carex lasiocarpa* (McClintock and Waterway 1993), 14 = *Carex pelita* (McClintock and Waterway 1993), 15 = *Podostemum ceratophyllum* (Philbrick and Crow 1992), 16 = *Filipendula rubra* (Aspinwall and Christian 1992), and 17 = *Allium vineale* (Ceplitis 2001).

populations do have lower clonal diversity, as one would expect. The difference between apomicts and aquatic plants could be due to ecological differences between their propagules (Lloyd 1984) and a lower establishment rate of apomicts. Apomicts have propagules (seeds) that are small, compared to those of aquatics, and these disperse vegetative fragments, turions, and so forth.

The prediction that rare species would be more clonal was borne out by analyses of three of the four indexes: *M*, *m* (fig. 4), and I_G (fig. 6*b*). Not all rare species have small population size (Rabinowitz 1981), though the association between rarity and small populations did seem to recur frequently in my sample and reduced population size is the likeliest cause of low genotypic diversity. In *Filipendula rubra* (Aspinwall and Christian 1992), a species that was classified as rare in the data set, the mean value of *G/N* was 0.58 in large populations but only 0.13 in small ones, a highly significant difference ($F_{1,21} = 12.77$, $P = 0.002$) that was independent of the

sex and clonal growth is general and selection for increased clonal growth is typical of small and marginal populations, the advantage may only be short-lived because small populations have a high probability of extinction (Henle et al. 2004; Matthies et al. 2004).

In passing, it is worth reflecting on what triggers the failure of sexual reproduction when vegetative (clonal) reproduction appears to be more robust. Of course, it is possible that this perception of failure is biased by the much greater attention that is given to sexual than to vegetative reproduction, but there is an intrinsic functional reason why sexual reproduction ought to be more vulnerable. Sexual reproduction can take place only in circumstances where vegetative growth is possible. However, this dependence is not symmetrical. Indeed, growth usually incurs a cost when sexual reproduction occurs (Silvertown and Dodd 1999). Hence, there are bound to be circumstances in which plants are physiologically capable of growth but not of reproduction, but there are no circumstances where the reverse is true. Plant vegetative growth and clonal reproduction are so closely linked (often involving the same organs) that it is almost inevitable that clonal reproduction will sometimes be physiologically possible even if sexual reproduction is not.

As predicted by Baker's law (Baker 1959), alien species had significantly lower values of G/N than natives (fig. 5c), though the other clonality indexes did not show a significant effect. Also as predicted, geographically marginal populations had lower values of G/N than populations in the hinterland of species' distributions (fig. 7), although this effect was only barely significant. No apomicts were present among the 17 species in my sample, but the association of apomixis with geographically marginal populations (geographic parthenogenesis) is already well established (Bierzychudek 1987; Peck et al. 1998; Horandl 2006) and occurs, for example, in the apomict *Antennaria rosea* (Bayer 1990). (This case was excluded from my sample because the source does not report values of G/N.)

Finally, I want to address possible sources of bias in this analysis. Major sources of bias that have concerned researchers in this field before, such as scale of sampling and type of genetic marker, were controlled within the analysis. The sample size used was large enough to include previously neglected factors such as life form and rarity and to compare types of clonality (apomixis vs. vegetative). However, the samples were still too small to analyze interactions among dependent variables. Phylogenetic effects (Silvertown and Dodd 1997) were eliminated in tests of two hypotheses, population age (1) and geographic range (5), by making comparisons within species, but phylogeny was not included in the other tests. A future phylogenetic reanalysis might produce results different from those reported here, though experience shows that phylogenetic and nonphylogenetic analyses often produce similar results (Ricklefs and Starck 1996).

The results of this analysis of more than 2,000 populations suggests that the ultimate clonal plant would be a rare, aquatic, alien apomict living in an undisturbed, geographically marginal habitat. This is such a restrictive set of ecological conditions that it is perhaps better regarded as a recipe for the failure of sexual reproduction than as clonal success. Revisiting the question that motivated this study, it is now clear that the ecological distribution of more extreme clonality tells us where sex fails, not why it persists. I suggest that clonal reproduction is not a substitute for sex but merely prolongs the time to extinction when sex is absent. In that case, genetic mechanisms, rather than any of the ecological factors I have examined, probably hold the answer to why sexual reproduction appears to be indispensable to long-term success.

Acknowledgments

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