

PATTERNS OF REPRODUCTIVE ISOLATION IN THREE ANGIOSPERM GENERA

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Abstract.—Analyses among animal species have found that reproductive isolation increases monotonically with genetic distance, evolves more quickly for prezygotic than postzygotic traits, and is stronger among sympatric than allopatric species pairs. The latter pattern is consistent with expectations under the reinforcement hypothesis. To determine whether similar trends are found among plant species, patterns of reproductive isolation (postpollination prezygotic, postzygotic, and “total” isolation) in three plant genera (*Glycine*, *Silene*, *Streptanthus*) were examined using data from previously published artificial hybridization experiments. In *Silene*, all measures of reproductive isolation were positively correlated with genetic distance. In contrast, in *Glycine* and *Streptanthus*, correlations between reproductive isolation and genetic distance were weak or nonsignificant, possibly due to the influence of biologically unusual taxa, variable evolutionary forces acting in different lineages, or insufficient time to accumulate reproductive isolation. There was no evidence that postpollination prezygotic reproductive isolation evolved faster than postzygotic isolation in *Glycine* or *Silene*. We also detected no evidence for faster accumulation of postmating prezygotic isolation between sympatric than allopatric species pairs; thus we found no evidence for the operation of speciation via reinforcement. In *Silene*, which included six polyploid species, results suggest that changes in ploidy disrupt a simple monotonic relationship between isolation and genetic distance.

Key words.—Genetic distance, plants, polyploidy, postzygotic, prezygotic, reinforcement, speciation.

Received September 4, 2003. Accepted February 9, 2004.

On comparing well-studied groups of higher plants with many groups of birds, mammals, and such insects as Drosophila, we find that discrete biological species are, in general, less frequent and other deviant species conditions are more characteristic in the plants. It follows that the causal factors determining the various modes of species organization should also be expected to differ in the two kingdoms. (Grant 1981, p. 59)

Throughout the twentieth century, extensive research capitalized on the experimental tractability of plants to examine relationships between reproductive barriers and other, frequently morphological, surrogates for phylogenetic distance between taxa. A consensus emerged from this “experimental taxonomy” that increasing reproductive isolation is generally associated with increasing differentiation in morphological and ecological traits (Stebbins 1950; Clausen and Hiesey 1958; Levin 1978; Vickery 1978; Grant 1981) and with geographical distance (Kruckeberg 1957). Occasional exceptions, however, indicated that morphologically indistinct groups can be strongly reproductively isolated (Ladizinsky and Abbo 1993) and that some anciently diverged taxa may retain interfertility (Parks and Wendel 1990). Other studies demonstrated the importance of chromosomal change in forming reproductive barriers, through either rearrangements or polyploidization (Stebbins 1971).

Although these general patterns in plant speciation are uncontroversial, their quantitative and qualitative comparability with patterns of speciation in other groups of organisms, particularly animals, is less well characterized. Numerous authors (Stebbins 1950; Clausen 1962; Levin 1979; Grant 1981; Gottlieb 1984; Arnold 1997) have emphasized the potential differences between plants and animals by pointing

to typical botanical characteristics that could strongly influence patterns of divergence and speciation. These botanical characteristics include uniparental reproduction through selfing or vegetative propagation, predominant hermaphroditism, indeterminate developmental systems, gametophytic gene expression, and a germ line that is not sequestered early in development (Stebbins 1999; Barrett 2001). For example, selfing may reduce the opportunity for selection to act on gamete or pollen discrimination traits, and therefore on traits involved in reproductive isolation (Levin 2000). Conversely, plants may be more tolerant of genetic perturbations resulting from ploidy changes, due to their relatively modular and indeterminate developmental systems (Stebbins 1950; Gottlieb 1984) or because they generally lack degenerate sex chromosomes (Orr 1990); selfing may further increase the likelihood of polyploid establishment (Ramsey and Schemske 1998). Accordingly, some mechanisms of speciation, such as those involving changes in ploidy or chromosomal rearrangements, might be vastly more common in plant than animal lineages (Lewis 1980; Orr 1990), while other mechanisms such as sexual selection or speciation via reinforcement might be less common (Levin 1970).

The classical plant literature is a rich but relatively unexploited source of data that may clarify whether patterns of reproductive isolation observed in animals also appear in plants. Three apparently common patterns are of particular interest here. First, analyses of the relationship between reproductive isolation and genetic distance in animal systems (Coyne and Orr 1989, 1997; Tilley et al. 1990; Gleason and Ritchie 1998; Sasa et al. 1998; Presgraves 2002; Mendelson 2003) have indicated that the magnitude of reproductive isolation between species pairs is generally positively associated with genetic distance, and therefore presumably with time since species divergence. Although classical plant studies have demonstrated the general association between reproductive isolation and differentiation in other—often quanti-

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tative—genetic traits (Clausen and Hiesey 1958; Levin 1978; Vickery 1978), the direct association between genetic distance and reproductive isolation has not been systematically examined in plant taxa.

Second, in a number of animal taxa, prezygotic isolation evolves faster than postzygotic reproductive isolation (Blair 1964; Prager and Wilson 1975; Coyne and Orr 1989; Gleason and Ritchie 1998; Mendelson 2003), indicating that prezygotic mechanisms may be more critical in the initial development of isolation between incipient animal species. The faster evolution of prezygotic mechanisms in animals has also been interpreted as evidence for strong sexual selection acting on prezygotic traits (Gleason and Ritchie 1998). However, in organisms such as plants where sexual selection may be weak or absent, expectations about the relative rates of evolution of different isolating mechanisms are unclear. In particular, it is presently unknown whether there are general differences in the rates at which prezygotic or postzygotic reproductive isolation evolves among plant taxa (Arnold 1997).

Finally, numerous studies suggest that speciation via reinforcement (Mayr 1963) may be widespread in animals. If selection for reinforcement is of general importance then prezygotic isolating mechanisms are expected to evolve more quickly between sympatric than allopatric species pairs (Coyne and Orr 1989). This pattern has been observed in *Drosophila* (Coyne and Orr 1989, 1997), as well as in 12 of 16 studies in animal species including frogs, fish, salamanders, and several insect taxa (reviewed in Hostert 1997). Patterns consistent with reinforcement have also been reported in some plant systems. In *Gilia*, crosses between sympatric taxa show much higher postpollination isolation than allopatric taxa (Grant 1966). In addition, there is evidence for flower color displacement, and/or increased self-compatibility or selfing rates, in the presence of congeneric species in *Phlox* (Levin and Kerster 1967; Levin 1985) and *Arenaria* (Fishman and Wyatt 1999), as well as evidence for changes in flowering times and selfing rates across strongly differentiated local environments such as mine boundaries in *Anthoxanthum* (Antonovics 1968). Although these provide possible evidence for reinforcement, the general prevalence of reinforcement in plants is unknown.

To explore the prevalence of these three patterns of reproductive isolation in plants, we analyzed data from previously published studies to investigate the relationship between reproductive isolation and genetic distance. Our first objective was to determine the relationship between genetic distance and reproductive isolation within each of three well-studied genera of angiosperms (*Glycine*, *Silene*, and *Streptanthus*). Moreover, because plant biologists have devoted considerable attention to speciation via polyploidization (Stebbins 1950; Soltis and Soltis 1999), and because changes in ploidy might radically and instantaneously reduce the ability of the derived polyploid to mate with its diploid progenitor(s) (Ramsey and Schemske 1998), we also evaluated the gross effect of changes in ploidy on the relationship between genetic distance and reproductive isolation in *Silene*—the genus in which data were available for polyploid species. Our second objective was to determine whether there are differences in the rates at which reproductive isolation evolves

prezygotically and postzygotically. Our third objective was to evaluate evidence for speciation via reinforcement by testing whether postpollination prezygotic isolating mechanisms evolve faster between sympatric than allopatric species pairs. Because the data we analyzed came from studies that evaluated isolation using experimental crosses, our analyses are limited to postpollination stages of reproductive isolation. Therefore, our measures of prezygotic isolation incorporate isolating mechanisms that act after pollination (mating) has occurred but do not include potential premating isolating factors such as pollinator behavior, flowering time, and ecological isolation.

METHODS

Sources of the Data

We analyzed previously published data on reproductive incompatibility and genetic distance among species in three plant genera: *Glycine*, *Silene*, and *Streptanthus*. Biological characteristics of these genera are outlined in Table 1. *Streptanthus* data included both intra- and interspecific taxon pairs within the *Streptanthus glandulosus* complex (Kruckeberg 1957; Mayer et al. 1994); taxon pairs in the two other genera primarily include comparisons between well-recognized species. These three genera were chosen because for at least 12 taxa within each group, data for genetic distance were available, and data for the degree of isolation were quantitative rather than qualitative (e.g., readily hybridizable). We only analyzed data from groups with 12 or more taxa to provide some statistical power for the evaluation of general trends. These criteria excluded many classical experimental taxonomic studies either because genetic distance data were not available or because changes in taxonomic designations obscured matches between classical data and published sequences. Overall, of 22 genera initially identified as potential data sources using literature searches in 1998 (data not shown; see Tiffin et al. 2001 for a partial list), only the three genera analyzed here met our criteria. A fourth genus, *Gossypium*, was excluded because of the difficulty of estimating genetic distances among multiple polyploid and reticulate species within this genus, and the many unresolved systematic relationships among taxa in this group (Seelanan et al. 1997, 1999). Allopatric and sympatric species pairs were determined using species ranges obtained from the literature or floras (*Glycine*: Brown et al. 1985; Grant et al. 1986; Harden 1990. *Silene*: Hitchcock and Maguire 1947; Prentice 1976; Ghazanfar 1981. All *Streptanthus* subtaxa were allopatric: Kruckeberg 1957). Following Coyne and Orr (1989, 1997), species pairs with ranges that overlapped at any point were classified as sympatric, whereas species with no known range overlap were classified as allopatric. In addition, for *Silene*, published chromosome numbers were used to classify species pairs as homoploid if they shared ploidy level or heteroploid if they did not (Hitchcock and Macguire 1947; Kruckeberg 1960; Prentice 1976; Ghazanfar 1989). The *Glycine* dataset analyzed here includes only one aneuploid taxon—*Glycine tomentella* (Shibles 1985); all *Streptanthus* subtaxa were homoploid (Kruckeberg 1957; Mayer et al. 1994). Data on reproductive incompatibility, genetic distances, and geographic

TABLE 1. Biological characteristics of the three analyzed plant genera.

	<i>Glycine</i>	<i>Silene</i>	<i>Streptanthus glandulosus</i> complex
Dominant life-history strategy	perennial herbaceous	perennial herbaceous	annual herbaceous
Predominant pollination strategy	insect and selfing	insect	insect
Self compatible	yes	yes (except dioecious spp)	yes
Frequency of selfing	low to high	low	low (except <i>S. niger</i>)
No. of species in genus	18	>300	N/A
Base chromosome number	20	12	14
Frequency of natural hybridization	rare	rare	N/A
Polyploid species	<i>G. tomentella</i> †	<i>S. boryi</i> <i>S. regia</i> <i>S. rotundifolia</i> <i>S. stellata</i> <i>S. vallesia</i> <i>S. virginica</i>	no
Domesticated taxa	<i>G. max</i> (soybean)	no	no
Number taxa included in analysis	12	26	19

† *Glycine tomentella* has diploid ($2n = 40$), tetraploid ($2n = 80$), and aneuploid ($2n = 38$) forms (Brown et al. 1985). The present analysis only includes data for diploid and aneuploid varieties (see online Appendix). *Glycine tabacina* also includes tetraploid races, however only data from diploid races were available for analysis.

range overlap are presented in the Appendix (available online at <http://dx.doi.org/10.1554/03-511.1.s1>).

Reproductive isolation

Reproductive isolation data for 191 taxon pairs came from a total of 13 published studies (see Appendix online) of artificial hybridization experiments that were conducted according to the same general procedures: plants were grown in a common environment, artificially pollinated by applying pollen from one species to the stigma of a second species (i.e., without interspecific pollen competition), and the degree of reproductive isolation was measured at several life-history stages. We analyzed three measures of reproductive isolation: prezygotic (postpollination), postzygotic, and total isolation. Postpollination prezygotic isolation was defined as the proportion of failed pollination attempts between species, relative to the average crossability within each parental species (i.e., $1 - (\% \text{ successful interspecific crosses}) / (\text{average } \% \text{ successful intraspecific crosses})$; McDade and Lundberg 1982). This stage of isolation primarily involves interactions between pollen and stigma, pistil, or gynoecium (De Nettancourt 1977), but might also include developmental stages immediately following fertilization but before any onset of seed or fruit formation is detectable. In 99 of 115 crosses (42/56 in *Glycine* and 57/59 in *Silene*) analyzed for prezygotic isolation, sufficient experimental detail was provided in the original studies (Prentice 1978; Ghazanfar 1981; Singh et al. 1987) to exclude the influence of such early postzygotic factors on this measure (e.g. the failure of pollen to germinate on stigmas or initiate fertilization was differentiated from the early formation of ‘bad’ seed; Prentice 1978); all data from the latter category were excluded from our analysis of postmating prezygotic isolation. As such, it is reasonable to expect that our measures of postmating prezygotic isolation primarily, if not exclusively, reflect prezygotic factors.

For all taxa, postzygotic isolation was defined as $1 - \% \text{ fertile pollen grains in the F1 hybrids}$. Finally, the combined effects of pre- and postzygotic isolation (i.e., total isolation)

were calculated as the linear sequential combination of prezygotic and postzygotic isolation measures (total isolation = pre + $(1 - \text{pre}) \times \text{post}$). All measures of isolation varied between 0 (no isolation) and 1 (complete isolation). When interspecific crosses did better than intraspecific crosses, reproductive isolation was set to zero (following Coyne and Orr 1989, 1997); this was observed infrequently (except for *Glycine falcata*—see Results) and only for estimates of prezygotic isolation.

Because both pre- and postzygotic isolation data were not available for every species pair, not all species were included in all analyses. In addition, for *Streptanthus*, only postzygotic reproductive isolation data were available. For all species, when data on reciprocal crosses were available, we averaged the reproductive isolation value of the crosses. Although the strength of both prezygotic and postzygotic isolation may differ depending on which species is used as a pollen parent and which is used as a seed parent (Tiffin et al. 2001), alternative ways of treating data from reciprocal crosses (e.g., taking the lowest or highest values of crossability) produced similar results. Finally, when isolation data for a given species pair were available from several studies, the average of the published isolation values (weighted by the number of crosses performed) was used.

Genetic distance

Genetic distance measures were chosen to maximize the number of species pairs for which we could obtain data. To meet this criterion, different measures of genetic distance were used for *Glycine* and *Silene* (DNA sequences) than for *Streptanthus* (allozyme). Pairwise genetic distances for *Streptanthus* were provided by M. Mayer (University of San Diego) and were calculated from 13 allozyme loci using Nei's unbiased electrophoretic genetic distance, D (Nei 1987; for details see Mayer et al. 1994). We calculated *Silene* and *Glycine* genetic distances from the internal transcribed spacer (ITS) regions 1 and 2, using sequences obtained from GenBank (see Appendix online). For each genus, sequences

were hand-aligned in Sequencher 4.1.2 (Gene Codes Corporation, Ann Arbor, MI), and pairwise genetic distances were calculated in PAUP* 4.0 (Swofford 1999), under a best fit model of molecular evolution selected using log likelihood ratio tests implemented in ModelTest 3.06 (Posada and Crandall 1998; for model details see online Appendix). To ensure that ITS alignment uncertainty did not unusually affect our estimates of genetic distance, we repeated our distance analyses using alignments generated by the automated alignment program ClustalX version 1.8 (Higgins and Sharp 1988; Thompson et al. 1997) under three alternative parameter values for gap opening and extension penalties. The resulting distances were strongly correlated with estimates generated from hand-alignments (data not shown), indicating that our results are robust to minor differences in sequence alignment. To verify our estimates of genetic distance, we also confirmed that they were substantially or wholly consistent with those in other published studies using alternative genetic markers (e.g., *Glycine*: Doyle et al. 1996; *Silene*: Oxelman et al. 1997; *Streptanthus*: Mayer and Soltis 1999). In particular, the six polyploid species included in the *Silene* dataset (see Table 1) are thought to be of allopolyploid origin (Heaslip 1951; Ghazanfar 1981); calculation of genetic distances between species pairs that include allopolyploid species may be problematic if distance is estimated from the DNA of just one of the two progenitor species of the allopolyploid. Nonetheless, our estimates of *Silene* genetic distances agree closely with prior analyses of nuclear DNA and chloroplast sequences (Oxelman et al. 1997; Burleigh and Holtsford 2003), as well as traditional taxonomic designations based on biogeography and morphology (Hitchcock and Maguire 1947). We note that incorrect phylogenies should only act to obscure whatever true correlations exist among reproductive isolation and phylogenetic relatedness.

Statistical Analyses

Correlations between reproductive isolation and genetic distance

Because some species were involved in multiple crosses, not all datapoints within each dataset are statistically independent. For this reason, we did not use standard parametric assessments of significance but instead used three alternative methods to assess the strength of relationship between reproductive isolation and genetic distance. First, to analyze the full dataset we used Mantel's test of matrix correlations—a conservative, permutation-based statistical test of association between distance matrices (Smouse et al. 1986; Manly 1997)—as used previously by Tilley et al. (1990) for similar analyses of patterns of differentiation in salamanders. Matrix correlations between observed genetic distance and reproductive isolation were calculated in Matlab (ver. 6.5, Math Works 1998). For each randomized permutation, genetic distance was held constant while the rows and columns of the corresponding matrix of reproductive isolation values were randomized, and a correlation coefficient calculated. Each dataset was subject to 1000 randomizations, generating a distribution of randomized coefficients against which the observed correlation coefficient was evaluated. Cases in which the observed coefficient was >95% of the randomized co-

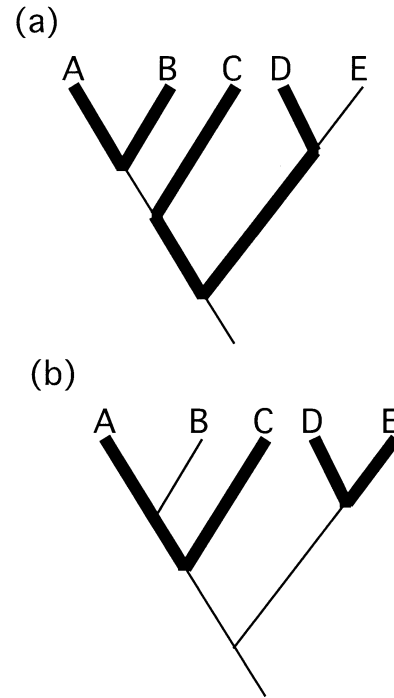


FIG. 1. A hypothetical phylogeny that illustrates two alternative sets, (a) and (b), of strictly phylogenetically independent species pairs. Evolutionary branches linking independent species pairs are shown in bold; note that no evolutionary branches are overlapping, and no single species is used more than once, within each set.

efficients were taken as evidence of a significant correlation between genetic distance and reproductive isolation. A one-tailed test was used because we assume that isolation is not negatively correlated with genetic distance.

Second, following Coyne and Orr (1989, 1997) we generated a reduced set of phylogenetically “corrected” species pairs for each dataset, using nested averaging to reduce all pairwise comparisons across each internal phylogenetic node to a single comparison. For example, in the hypothetical phylogeny shown in Figure 1, the complete set of corrected distances would be AB, DE, $(CA + CB)/2$, and $\{[(DA + DB)/2 + DC]/2 + [(EA + EB)/2 + EC]/2\}/2$ (see also Fitzpatrick 2002). Third, where sufficient data were available (i.e. for at least five independent species pairs), a set of strictly phylogenetically independent pairwise comparisons (see Felsenstein 1985) were identified; for example, Figure 1a and 1b depicts two alternative sets of strictly independent pairs that can be generated from a hypothetical phylogeny. Evolutionary branches linking these species pairs are shown (in bold) to be nonoverlapping and no single species is used in the generation of more than one independent datapoint. Additional alternative sets of independent pairs that could be generated from the same phylogeny include AB and DE, or BC and DE. For the analysis in this paper, strictly independent species pairs were selected in each genus to maximize the number of pairs that could be obtained from the available nonindependent data; alternative sets of strictly independent pairs produced results negligibly different from those presented (data not shown). Though both these methods correct for statistical and phylogenetic nonindependence between da-

tapoints while reducing the sample size of a dataset, strict phylogenetic independence is a more conservative criterion for generating independent data from a matrix of nonindependent species pairs. Both corrected and strictly independent datasets were used to assess the nonparametric (Kendall's τ) rank correlation between reproductive isolation and genetic distance, in JMP (ver. 3.1, SAS Institute 1995). We used these multiple alternative methods because there is ongoing discussion on how best to analyze these kinds of data in general (e.g. Legendre et al. 1994; Fitzpatrick 2002) and these patterns specifically (e.g. Coyne and Orr 1989; Tilley et al. 1990; Mendelson 2003); we assume that patterns resulting from underlying biological processes will be generally robust to any particular method of analysis.

Comparisons of evolutionary rates

Although we have no direct calibration of evolutionary rates in our three plant genera, we used genetic distance as a proxy for time since divergence to examine relative rates of evolution of each stage of reproductive isolation, within and among genera. The mean rate at which isolation evolved was estimated as the regression coefficient of a regression of reproductive isolation on genetic distance; the regression was constrained through the origin as we assume species start off as populations that are genetically identical and reproductively compatible (Hillis et al. 1990). Because we have little a priori information about the expected form of this regression, both linear (least squares) and logistic (maximum likelihood) regression models were used. The logistic model is attractive because the dependent variable is bounded by zero and one, as are our indices of reproductive isolation, and isolation values can be interpreted as probabilities of failure in each interspecific cross—data for which the logistic regression is appropriate (Sokal and Rohlf 1995). Both linear and logistic models produced qualitatively similar results and the same substantive conclusions when comparing relative rates of evolution of isolation. We note that, if there is substantial disagreement between our intertaxon genetic distances and their relative divergence times, our proxy for measures of rate may not accurately estimate the pace at which isolation has accumulated.

For *Glycine* and *Silene*, permutations were used to test for significant differences in relative evolutionary rates (regression coefficient estimates) between (1) sympatric and allopatric species pairs, and (2) pre- and postzygotic isolation. For *Silene*, we also compared relative rates in homoploid versus heteroploid species pairs. The permutation tests were conducted by combining data from the two groups being compared into a single dataset and then resampling these combined data to create 1000 pseudodatasets. Each pseudodataset contained two groups with sample sizes equal to the sizes of the original groups. The absolute difference in evolutionary rates (regression coefficients) between the two groups was then calculated, generating a distribution of 1000 pseudorandom rate differences. The observed difference in rates between the actual groups was then compared with this distribution; cases in which the observed difference was greater than 95% of the resampled values were taken as evidence of a significant difference between the observed rates.

We used a similar resampling approach to compare rates of evolution between *Glycine* and *Silene* at each stage of reproductive isolation; these comparisons were limited to these two genera because *Streptanthus* involved different estimates of genetic distance. All rate comparisons were implemented in Matlab (ver. 6.5, Math Works 1998).

RESULTS

Relationship between Reproductive Isolation and Genetic Distance

The strength of association between isolation and genetic distance differed quantitatively and qualitatively between the three genera (Fig. 2). Reproductive isolation and genetic distance were significantly positively correlated in the two of the three genera (*Glycine* and *Silene*; Table 2), although results in *Glycine* were dependent upon the method of correcting for phylogenetic dependencies with significant correlations detected for the phylogenetically corrected data only (Table 2). In contrast, the correlation between postzygotic isolation and genetic distance in *Streptanthus* was not significant for all analyses (Fig. 2; Table 2).

In *Glycine*, the strength of association between postmating prezygotic reproductive isolation and genetic distance appears to be weakened by the influence of six species pairs with genetic distances greater than 0.15 but little or no accompanying reproductive isolation (Fig. 2a). All six of these pairs involve *G. falcata*. In addition, in half (5 of 10) of the crosses involving *G. falcata*, the success of intraspecific pollinations was lower than the success of interspecific pollinations (Broue et al. 1982; Singh et al. 1987; see Appendix online). When *G. falcata* is removed from the analysis, the matrix correlation between genetic distance and prezygotic reproductive isolation is strengthened considerably ($r = 0.539$, $P = 0.052$; Table 2). Removing *G. falcata* from the phylogenetically corrected dataset also strengthens the correlation between postpollination prezygotic isolation and genetic distance ($r = 0.669$, $P = 0.007$; Table 2) although the effect is less, presumably because the phylogenetic correction minimizes the impact of multiple comparisons.

Regardless of the influence of *G. falcata*, we observed only marginal or nonsignificant correlations between both postzygotic and total isolation with genetic distance in *Glycine* (Table 2). This was the case, even though reasonably large correlation coefficients were observed (Table 2) and the patterns of isolation clearly suggest that isolation increases with genetic distance (Fig. 2). Because we had smaller sample sizes for postzygotic and total than prezygotic isolation analyses, the lack of significance may be due to lower statistical power. Although there are few established protocols for formal power analyses of randomization tests (Efron and Tibshirani 1993; Manly 1997) we used bootstrap resampling of the observed data to determine the 95% confidence intervals around the observed correlation coefficients and thus a rough estimate of the power to detect significant correlations in the uncorrected *Glycine* dataset (Efron and Tibshirani 1993). These confidence intervals were large (prezygotic: 0.306–0.734; postzygotic: 0.491–0.854; total: 0.412–0.749; bootstrap replicates = 1000; data with *G. falcata* removed) especially in comparison to those determined for *Silene* (pre-

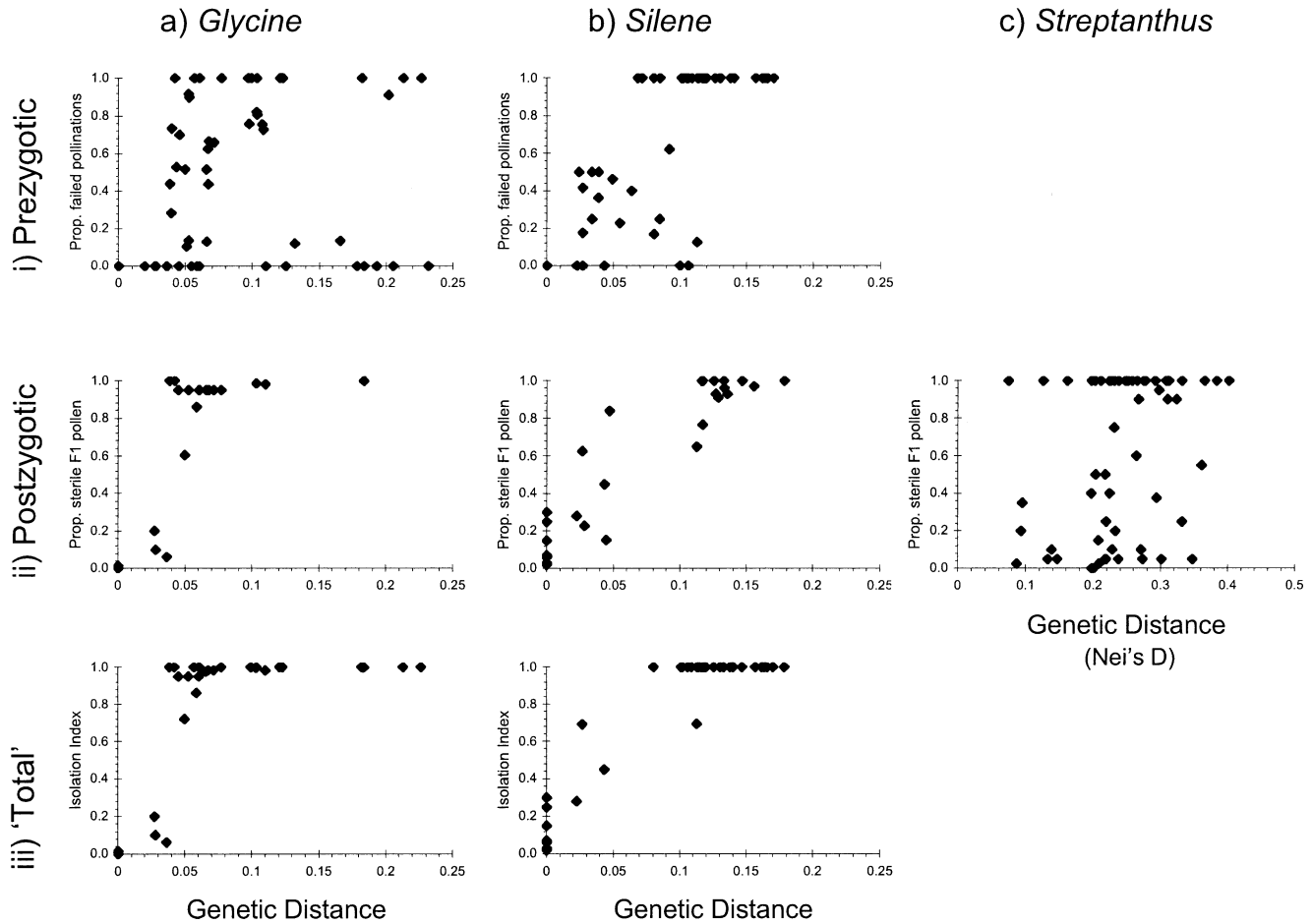


FIG. 2. Strength of reproductive isolation versus genetic distance between taxa in the genera (a) *Glycine*, (b) *Silene*, and (c) *Streptanthus*. Three categories of reproductive isolation are (i) prezygotic, (ii) postzygotic, and (iii) total isolation. Statistics for each correlation are given in Table 2.

zygotic: 0.677–0.878; postzygotic: 0.842–0.940; total: 0.859–0.936) and suggest that large variance in our uncorrected *Glycine* dataset, perhaps inflated by small sample size, may be partially responsible for the nonsignificant correla-

tions. With respect to the phylogenetically independent analyses of *Glycine*, power tests are likely inadequate for data with sample sizes <10 (Sokal and Rohlf 1995). Nonetheless, we note that for the values of Kendall's τ observed in *Glycine*,

TABLE 2. Correlations between reproductive isolation and genetic distance for three plant genera. Correlations were calculated separately on data from postmating prezygotic, postzygotic, and total fitness measurements; N , total number of species pairs analyzed; N_{spp} , number of unique taxa used in crosses. Each correlation is evaluated with three alternative approaches. Full (uncorrected) datasets were analyzed with Mantel matrix permutation tests (Smouse et al. 1986): Correl, pairwise matrix correlation (Manly 1997); P , value from Mantel test (number of values exceeding the observed correlation coefficient/1000). Corrected and strictly phylogenetically independent datasets were evaluated with Kendall's nonparametric rank correlation: Correl, Kendall's τ ; P , significance value.

Taxon	Dataset/analysis	Postmating prezygotic isolation				Postzygotic isolation				Total isolation			
		N	N_{spp}	Correl	P	N	N_{spp}	Correl	P	N	N_{spp}	Correl	P
<i>Glycine</i>	full dataset	56	12	0.279 ¹	0.181	20	11	0.688	0.150	29	12	0.586	0.210
	corrected	12	12	0.597 ²	0.010	11	11	0.654	0.006	11	11	0.525	0.030
	strictly independent	6	12	0.640	0.075	5	8	0.600	0.142	5	10	0.600	0.142
<i>Silene</i>	full dataset	59	22	0.789	0.105	29	18	0.901	0.001	41	18	0.904	0.014
	corrected	10	22	0.598	0.018	8	18	0.618	0.034	9	18	0.546	0.060
	strictly independent	6	12	0.596	0.102	6	12	0.867	0.015	5	10	0.949	0.023
<i>Streptanthus</i>	full dataset					61	19	0.3861	0.209				
	corrected					13	19	0.337	0.111				
	strictly independent					6	12	0.358	0.329				

¹ Matrix correlation with *G. falcata* removed is $r = 0.539$, $P = 0.052$; see text.

² Rank correlation with *G. falcata* removed is $\tau = 0.669$, $P = 0.007$; see text.

TABLE 3. Correlation and rate differences between homoploid and heteroploid species pairs in *Silene*. Correlations were evaluated with Mantel matrix permutation tests (Smouse et al. 1986); Correl, pairwise matrix correlation (Manly 1997); P , number of values exceeding the observed correlation coefficient/1000. Rate was estimated as the slope of the regression of reproductive isolation on genetic distance, under both linear and logistic regression models. Comparisons between regressions were made using a randomization test in which the observed difference between slopes was compared to a distribution of differences generated from 1000 pseudorandom datasets; N , number of species pairs used in crosses; P , value determined from randomization test (number of values exceeding the observed difference in slopes/1000).

Isolation stage	Ploidy type	N	N_{spp}	Correlations		Rate comparisons			
				Correl	P	Linear model	P	Logistic model	P
Prezygotic	homo	50	20	0.802	0.121	7.35	0.206	13.03	0.308
	hetero	10	10	0.631	0.246	7.17		18.82	
Postzygotic	homo	27	18	0.900	<0.001	7.24	‡	16.45	‡
	hetero	2	4	†	‡	6.49†		210.14†	
Total	homo	29	18	0.907	0.017	7.63	0.183	28.70	0.164
	hetero	10	11	†	‡	7.40†			

† All isolation values = 1.

‡ Unable to evaluate statistically.

at least one to three additional observations would be necessary to exceed the critical value required for $\alpha < 0.05$ (Rohlf and Sokal 1981).

High variance and small sample size may also have limited our ability to detect a significant correlation in the *Streptanthus* postzygotic dataset (e.g. the bootstrap confidence interval is 0.166–0.587 for the complete *Streptanthus* dataset). However, unlike *Glycine*, the *Streptanthus* dataset is thought to primarily include intra- and subspecific taxon pairs (according to classifications based primarily on morphological differentiation; Kruckeberg 1957; Mayer and Soltis 1999) so the lack of association between isolation and distance in this genus could also result from insufficient time to accumulate genetic barriers between subspecific taxa or to develop the expected association between genetic divergence and reproductive isolation (see Discussion). Although phenotypic classifications (Kruckeberg 1957) indicate that the *Streptanthus* taxon pairs are much younger (more recently derived) than our *Silene* and *Glycine* taxa, because different measures of genetic distance were used in our primary analyses, we were unable to evaluate this with a direct comparison of mean genetic distance between all 19 *Streptanthus* taxa and the taxa in the other two genera. As an alternative, we generated a rough estimate of the calibration between ITS and allozyme (Nei's D) genetic distances in *Streptanthus*, by obtaining previously published ITS 1 and 2 sequence data for a subset of 10 of the 19 taxa used in our primary analyses (see Appendix) and using these to generate a matrix of genetic distances with the same phylogenetic procedures outlined in the Methods. A regression of ITS genetic distance against allozyme D for these 10 species produced a best-fit line of [ITS = $-0.002 + 0.1148 \times$ Allozyme] ($r^2 = 0.564$, $P < 0.0001$). Using this rough conversion between ITS genetic distance and Nei's D , we estimate that our *Streptanthus* taxon pairs are, on average, diverged by an ITS distance of 0.0298 (range: 0.0069–0.0630), in comparison to the average intertaxon ITS distance of 0.0769 (range: 0.0–0.2317) and 0.0849 (range: 0.0–0.1785) in *Glycine* and *Silene*, respectively. This suggests that our *Streptanthus* taxa are indeed younger than the species in the other two genera.

An alternative hypothesis for the lack of observed corre-

lation in *Streptanthus* is that the allozyme variants used to generate Nei's D have been influenced by selection, so that this measure of genetic distance provides a poor estimate of divergence times. Therefore, for the 10 *Streptanthus* taxa for which we obtained ITS sequences, we also evaluated the strength of association between postzygotic reproductive isolation and ITS (noncoding sequence) genetic distance. The resulting matrix correlation between ITS genetic distance and postzygotic isolation was both very small and nonsignificant ($r = 0.0261$, $P = 0.662$, $N = 21$, $N_{spp} = 10$), suggesting that postzygotic isolation may not be significantly correlated with genetic distance among these *Streptanthus* taxa, regardless of which measure of genetic distance is used.

Influence of Changing Ploidy on Reproductive Isolation

To assess the effect of ploidy changes on the relationship between isolation and genetic distance, we examined differences between heteroploid and homoploid species pairs in *Silene*, which included six tetraploid species. Reproductive isolation between species with the same ploidy level was positively correlated with genetic distance although this association was not significant for prezygotic isolation (Table 3). In contrast, species pairs with different ploidy levels (heteroploid species pairs) were almost all completely reproductively isolated (Table 3) precluding statistical analysis for both postzygotic and total isolation measures. Nonetheless, we detected no significant differences in rates of evolution of reproductive isolation between homoploid species pairs compared to heteroploid species pairs (Table 3; postzygotic isolation was not evaluated due to few heteroploid observations).

Although these results suggest that changes in ploidy might disrupt a simple monotonic relationship between isolation and genetic distance, there are at least two reasons that these comparisons should be viewed with some caution. First, the range of genetic distances spanned by heteroploid species pairs (0.10–0.178) is considerably more narrow than that of homoploid species pairs (0.023–0.170); in particular, there were no young heteroploid species pairs included, so that the rate estimate for this group may underestimate the actual rate

TABLE 4. Rates of evolution of prezygotic versus postzygotic reproductive isolation in two plant genera (*Glycine* and *Silene*). Rate of change in reproductive isolation is estimated as the slope coefficient (b) of a regression of reproductive isolation on genetic distance. Both linear and logistic regression models were evaluated. Rate comparisons between groups were made using a randomization test in which the observed difference between slope coefficients was compared to a distribution of differences generated from 1000 pseudorandom datasets; N , observation sample size; N_{spp} , number of unique taxa used in crosses; P , number of randomized differences exceeding the observed difference in regression coefficients/1000. Results are given for the *Glycine* dataset with *G. falcata* both included and excluded. Prezygotic data were not available for *Streptanthus*.

Taxon	Stage	N	N_{spp}	Rate (linear model)	P	Rate (logistic model)	P
<i>Glycine</i>	prezygotic	56	12	4.04	0.053	0.58	0.094
	postzygotic	20	11	10.35		29.82	
<i>Glycine</i> (no <i>G. falcata</i>)	prezygotic	46	11	7.18	0.101	4.23	0.154
	postzygotic	19	10	12.82		29.75	
<i>Silene</i>	prezygotic	59	22	7.30	0.0268	14.24	0.602
	postzygotic	30	18	7.10		17.60	

of accumulation of isolation during the earliest stages of divergence. Second, polyploidy may affect the observed relationships between genetic distance and isolation by obscuring genetic distance measures. In particular, measurements of genetic distance in allopolyploids may be obscured if distance is estimated from the DNA of just one of the two progenitor species, whereas the degree of reproductive isolation may be determined by the genetics of only one, or both, progenitor species. Despite this possibility, we find considerable phylogenetic congruence between our genetic distance measures and relationships inferred from different markers in other studies, and these relationships are consistent with biogeographic patterns and morphological trait groupings in *Silene* (see Methods). In addition, the relatively strong correlations between reproductive isolation and genetic distance in *Silene* (Fig. 2; Table 2) are an unexpected result if our estimates of genetic distance involving polyploid species (six of 27 species, and 17% of all species pairs) are substantially incorrect; this suggests that our measures have not been heavily biased by basing our estimates of genetic distances on only one of two homeologous sequences in polyploid species.

Relative Rates of Postpollination and Postzygotic Isolation within and between Genera

Data on prezygotic and postzygotic isolation were available for *Silene* and *Glycine*. We detected no difference between rates of evolution at these two stages in *Silene*, whereas postzygotic isolation appeared to evolve significantly faster than prezygotic isolation in *Glycine* (Table 4). As noted above, however, *Glycine falcata* contributes to many low prezygotic isolation, high genetic distance species pairs. Removing *G. falcata* resulted in a nonsignificant difference between pre- and postzygotic isolation (Table 4) suggesting that this species may exaggerate the observed difference in rates between pre- and postzygotic isolation in the full dataset.

Because *Glycine* and *Silene* have comparable measures of reproductive isolation and genetic distance, we were able to evaluate the relative rates of evolution at three stages of isolation between these two genera. When the full *Glycine* dataset was used, *Silene* appeared to evolve postpollination prezygotic isolation faster than *Glycine*, however once *G. falcata* was removed this rate difference was not significant

(Table 5), similar to our finding for pre- versus postzygotic isolation within *Glycine*. No differences were detected in the rates of accumulation of postzygotic or total isolation between *Glycine* and *Silene* (Table 5). This finding suggests that genera of roughly the same age (based on mean ITS genetic distance) and that share a number of important life-history characters (see Table 1) might evolve isolation at similar average rates, even if the strength of association between reproductive isolation and genetic distance differs between groups.

Evidence for Speciation via Reinforcement

We examined evidence for reinforcement in *Glycine* and *Silene* by comparing rates of evolution of reproductive isolation between sympatric and allopatric species pairs. (*Streptanthus* was not analyzed because all taxa were allopatric; Kruckeberg 1957; Mayer and Soltis 1999.) We detected no evidence that postmating prezygotic isolation evolved at faster rates between sympatric than allopatric species pairs in either genus (Fig. 3; Table 6). This finding is inconsistent with expectations if reinforcement is operating at postpollination prezygotic stages in these two plant groups.

DISCUSSION

Relationship between Reproductive Isolation and Genetic Distance

Our analyses provide a set of ‘‘rough portraits’’ (Presgraves 2002) of the relationship between reproductive isolation and genetic distance between species in three diverse angiosperm genera. These rough portraits indicate that some patterns of reproductive isolation in plant genera are generally consistent with those found in animal taxa to date, although some can depart markedly. Prior analyses in animal systems, including *Drosophila* (Coyne and Orr 1989, 1997), amphibians (Sasa et al. 1998), and moths and butterflies (Presgraves 2002), have detected positive correlations between reproductive isolation and genetic distance. Our analyses of plant genera reveal a more varied set of relationships ranging from consistently large positive correlations (*Silene*) to associations that are weak or absent (*Streptanthus*). Findings for *Silene* agree most closely with the general observation in

TABLE 5. Relative rates of evolution of reproductive isolation in *Glycine* versus *Silene*. Rate was estimated as the slope coefficient (b) of a regression of reproductive isolation on genetic distance, under both linear and logistic regression models. Comparisons between regressions were made using a randomization test in which the observed difference between slopes was compared to a distribution of differences generated from 1000 pseudorandom datasets; N , sample size; N_{spp} , number of unique taxa used in crosses; P , value determined from randomization test (number of values exceeding the observed difference in slopes/1000). *Glycine* results are given with *G. falcata* both included and excluded.

Stage	Taxon	N	N_{spp}	Rate (linear model)	P	Rate (logistic model)	P
Prezygotic	<i>Glycine</i>	65	12	4.04	0.067	0.58	0.052
	<i>Silene</i>	59	22	7.30		14.24	
Prezygotic (no <i>G. falcata</i>)	<i>Glycine</i>	54	11	7.18	0.552	4.23	0.572
	<i>Silene</i>	59	22	7.30		14.24	
Postzygotic	<i>Glycine</i>	22	11	10.35	0.323	29.82	0.365
	<i>Silene</i>	30	18	7.10		17.60	
Total	<i>Glycine</i>	32	12	7.92	0.181	38.09	0.121
	<i>Silene</i>	53	18	7.54		32.48	

animal taxa that isolating barriers increase in concert with increasing phylogenetic distance. The observed correlations between isolation and genetic distance indicate that accumulation of barriers to reproduction might be more gradual (or clocklike; Presgraves 2002) in this genus. This pattern might also indicate that reproductive isolation is due to many genes of small effect (Coyne and Orr 1998; Edmands 2002), although identifying the genetic basis of reproductive isolation clearly requires extensive work well beyond the scope of this investigation.

In contrast, the weak or marginal correlations observed in *Glycine* and *Streptanthus* suggest that isolation does not always evolve in a clocklike manner among plant taxa, perhaps

because few genes of large effect or changes in chromosomal architecture underlie isolation (Edmands 2002), or because different evolutionary forces act on isolation mechanisms in different lineages. Although the lack of significant correlations in *Glycine* might also be due to low statistical power, a general decoupling of cpDNA divergence from morphological and ecological patterns of diversification (Doyle et al. 1990), as well as disagreement between some morphological boundaries and variation at the nuclear histone H3-D locus (Doyle et al. 1999), have previously been noted in this genus. Our findings similarly suggest a complex relationship between phylogenetic relatedness and groupings defined by reproductive compatibility in *Glycine*.

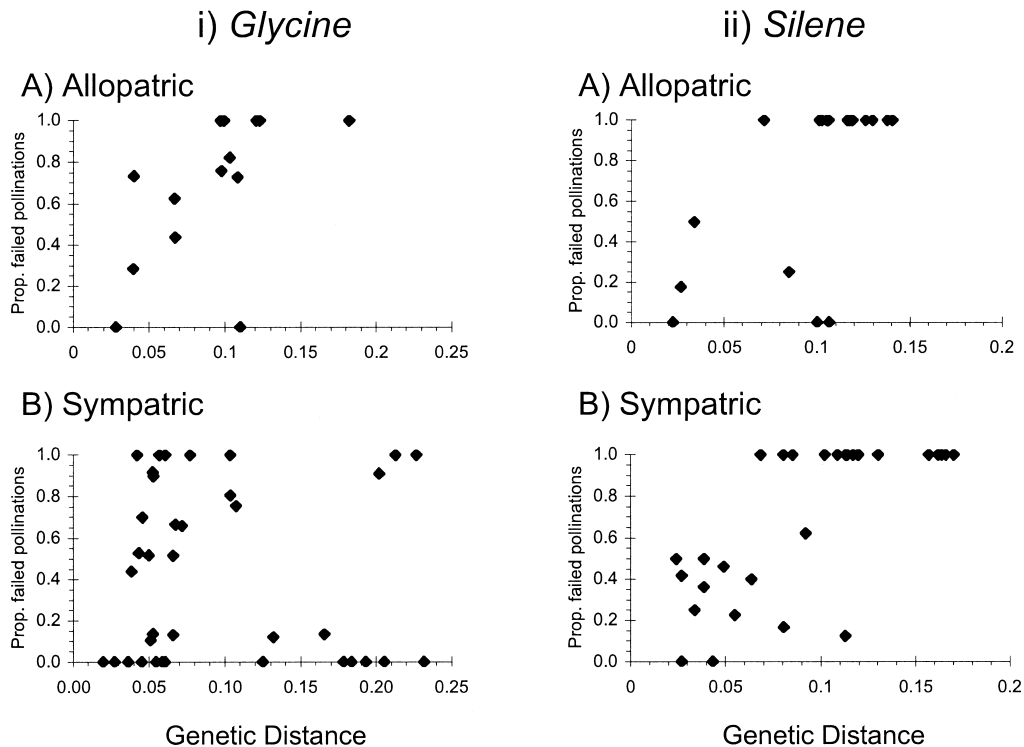


FIG. 3. Strength of postpollination prezygotic reproductive isolation over genetic distance for allopatric versus sympatric species pairs in (i) *Glycine* and (ii) *Silene*. No differences were detected in either genus; statistics are given in Table 6.

TABLE 6. Relative rates of evolution of reproductive isolation among sympatric and allopatric species pairs. Rate was estimated as the slope coefficient (b) of a regression of reproductive isolation on genetic distance, under both linear and logistic regression models. Comparisons between allopatric and sympatric isolation were made using a randomization test in which the observed difference between slopes was compared to a distribution of differences generated from 1000 pseudorandom datasets; N , sample size; N_{spp} , number of unique taxa used in crosses; P , value determined from randomization test (number of values exceeding the observed difference in slopes/1000). Results with *Glycine falcata* excluded do not differ substantively from those presented. Data for *Streptanthus* were not available (all populations are allopatric).

Taxon	Isolation stage	Allopatric/ Sympatric	N	N_{spp}	Rate (linear model)	P	Rate (logistic model)	P
<i>Glycine</i>	prezygotic	sympatric	40	10	3.22	0.221	-1.89	0.643
		allopatric	15	10	6.99		10.90	
	postzygotic	sympatric	15	8	10.75	0.372	32.12	0.275
		allopatric	3	4	9.03		21.77	
	Total	sympatric	21	10	8.04	0.399	40.75	0.773
	allopatric	7	8	7.68	30.37			
<i>Silene</i>	prezygotic	sympatric	30	18	7.20	0.502	14.25	0.524
		allopatric	19	18	7.45		14.22	
	postzygotic	sympatric	14	11	6.90	0.254	16.30	0.204
		allopatric	5	9	8.16		24.04	
	Total	sympatric	20	14	7.17	0.224	30.56	0.445
		allopatric	11	13	8.96		38.04	

Our analyses in *Glycine* also highlight the influence that individual species with unusual crossing behavior can exert on overall patterns of reproductive isolation within a genus, and the utility of these analyses in identifying such taxa. *Glycine falcata* has been consistently recognized as an unusual member of its genus, on the basis of numerous autapomorphic morphological, mating, and biochemical features (Hermann 1962; Mies and Hymowitz 1973; Hymowitz and Singh 1987; Doyle et al. 1990; Singh et al. 1992; Kollipara et al. 1997). For example, *G. falcata* is a primarily cleistogamous species with high selfing rates that are exaggerated by the presence of subterranean inflorescences—a unique morphological feature among the *Glycine* species we analyzed (Doyle et al. 1990; Singh et al. 1992). Although the basis of its unusual crossing behavior is unknown, one possibility is that high rates of selfing have led to the fixation of deleterious alleles in *G. falcata*, contributing to the expression of heterosis in interspecific crosses and obscuring the expression of reproductive isolation at postpollination prezygotic stages. Regardless, it is clear that this species exhibits novel crossing behavior that, along with its many other unique traits (Doyle et al. 1996), might be the product of unusual evolutionary mechanisms or forces.

In contrast to *Glycine*, in *Streptanthus* our failure to detect a significant correlation between isolation and genetic distance may simply be a function of insufficient time to accumulate reproductive isolation between taxon pairs or to develop the expected association between reproductive isolation and intertaxon genetic divergence. Our *Streptanthus* taxon pairs appear to be considerably younger than species analyzed in the other two genera, and the high variance in the relationship between genetic distance and reproductive isolation in *Streptanthus* could result from the relatively recent differentiation of its taxa. This may be a common pattern among recently derived taxa. For example, isolation values from thirteen intra- and interspecific crosses among rice cultivars and wild *Oryza* species failed to show any association with chloroplast DNA divergence (Edmands 2002; see this

review for other examples). Similarly, in their analysis of ethological isolation among *Desmognathus* salamanders with Nei's D genetic distances ranging from 0.043 to 0.643, Tilley et al. (1990) found that genetic distance itself was a poor predictor of reproductive isolation, once the influence of geographic distance on both these measures of divergence was removed.

Polyploidy and the Evolution of Reproductive Isolation

While the majority of our species pairs involved crosses within ploidy, polyploid species within *Silene* enabled us to evaluate the gross effects of ploidy changes on the expression of reproductive isolation and its relationship with genetic divergence. Changes in ploidy are expected to produce strong isolation over short genetic distances because crosses between taxa that differ in ploidy levels may suffer from abnormal dosage effects, meiotic disruption, and other consequences of unbalanced karyotypes (Ramsey and Schemske 1998; Levin 2000). Although our analyses were limited, our results are generally consistent with these expectations. The majority of heteroploid species pairs were substantially or completely reproductively isolated, whereas the expression of isolation among homoploid pairs was more varied and more closely associated with genetic divergence (Table 5). Nonetheless, the absence of young heteroploid species pairs makes drawing stronger conclusions difficult; clearly, rapid rates of isolation among young species cannot be detected if no young species pairs are represented in the analysis. Data for recently derived polyploids would provide an opportunity to assess the supposition that ploidy changes generally produce rapid changes in reproductive isolation over short genetic distances, that is, "instantaneous" speciation (Stebbins 1950; Lewis 1980).

Evolutionary Rates between Stages of Isolation, and between Genera

In animal taxa, prezygotic isolation has been found to evolve faster than postzygotic isolation (Blair 1964; Prager

and Wilson 1975; Coyne and Orr 1989; Gleason and Ritchie 1998; Mendelson 2003), a difference attributed to different selective forces acting on the two stages of isolation; loci contributing to postzygotic isolation are likely evolving due to drift or indirect selection alone, whereas prezygotic traits may also be subject to direct selection (especially sexual selection), which can speed the overall rate at which prezygotic isolation accumulates (Gleason and Ritchie 1998). However, we detected no evidence for significant rate differences between these stages of reproductive isolation (once the influence of *G. falcata* was accounted for in *Glycine*). In fact, rate estimates for postzygotic isolation were almost always larger than estimates for prezygotic isolation (Table 4; see also Fig. 2). This apparent difference between plants and animals might be due to the fact that our data were collected from studies that did not include premating components of prezygotic isolation, including pollinator behavior, flowering time, and ecological isolation. These prepollination mechanisms can act as important barriers to gene exchange among plants (Levin 1978; Grant 1994) and may more closely resemble the premating (ethological) stages analyzed in many animal studies (Hostert 1997).

Evidence for Speciation via Reinforcement

A final contrast between our findings and those in animal studies is the lack of evidence for reinforcement. Evidence for reinforcement is common in many recent studies of isolation patterns in animals (Hostert 1997), whereas we found no indication that prezygotic isolation evolves faster among sympatric as opposed to allopatric taxa. These findings are consistent with classical arguments that reinforcement may be less effective and less important in plants (especially predominantly insect-pollinated, perennial species such as those analyzed here; Table 1) than in animals. The reasoning underlying these arguments is twofold. First, for reinforcement to be successful, selection against heterospecific matings must operate in the parental generation (Grant 1981). Such selection may be relatively ineffective in plant species that rely on insect pollinators; if pollinators are insufficiently constant in discriminating between different floral types then selection to reinforce characters that favor homotypic matings will be weak (Grant 1981, 1994). This explanation is unlikely to account for our findings, however, which apply only to postpollination isolating mechanisms. (Note that, although none are included in our analyses, this argument also does not apply to wind-pollinated species.) Second, it has been proposed that heterospecific matings may be insufficiently costly to drive the process of reinforcement in plant systems (Levin 1970; Grant 1981). In particular, even after interspecific fertilization has occurred, angiosperms are routinely able to manipulate resource provisioning of developing embryos to minimize or prevent investment in hybrid offspring (Levin 1970). As such, interspecific hybridization per se need not substantially reduce lifetime fitness, especially in perennial species that can redirect resource investment in offspring to future reproductive opportunities (Grant 1981).

There are also at least two methodological considerations that might have influenced our failure to find evidence for reinforcement: the artificial hybridization experiments from

which our data were collected did not assay isolation at pre-pollination stages or examine the possible influence of interspecific pollen competition on postmating prezygotic isolation. In the latter case, conspecific pollen precedence could potentially be influential as an isolating mechanism among sympatric taxa. However, although numerous empirical manipulations have shown that pollen competition can be important in determining the success of intraspecific pollination (Skogsmyr and Lankinen 2002), there are limited data on the role of pollen competition in isolating different species, especially when plants are naturally pollinated (Howard 1999; though see Ramsey et al. 2003). In addition, Coyne and Orr (1989, 1997) found no difference between the relative degree of isolation among *Drosophila* species measured with and without interspecific sperm competition (Coyne and Orr 1989), suggesting that, in this animal group at least, male gamete competition does not substantially influence the observed patterns of prezygotic reproductive isolation.

Equally, prepollination isolating mechanisms, such as flowering time shifts, pollinator preferences, or ecological specialization, could be very important in maintaining effective isolation between plant species (Levin 1978; Grant 1994; Schemske 2000), and our findings do not rule out the possibility that reinforcement has acted on such traits in these taxa. If reinforcement is operating at these stages, the expectation among sympatric species pairs would be an enhanced frequency of character displacement, such as local habitat differentiation or nonoverlapping reproductive (e.g. flowering) timing, as a result of past selective pressure for avoidance of heterospecific matings. The prevalence of reinforcement speciation in plants remains particularly underexamined in this regard. Nonetheless, our results indicate that reinforcement does not seem to be operating on (noncompetitive) postmating prezygotic isolation in *Glycine* and *Silene*, a stage that has been demonstrated to play an important role in divergence and speciation processes (e.g., Levin 1978; Arnqvist et al. 2000) including speciation via reinforcement (Servedio 2001).

Prospects

Our results provide evidence that patterns of reproductive isolation may differ considerably among plant groups as well as between some plant and animal taxa. This variation is consistent with the recent suggestion that there is considerable variety in patterns of acquisition of reproductive isolation among taxonomic groups, and that reproductive incompatibility need not be straightforwardly predictable from parental divergence (Edmands 2002). Moreover, it suggests that there may be much to learn about the evolution of reproductive isolation by extending analyses of this kind to additional genera from diverse biological groups, including (but not limited to) both plants and animals.

Our analysis also points to a number of avenues of research that remain underexplored, especially in plants. In particular, more studies that examine premating isolation mechanisms among multiple closely related species are essential to comprehensively assess relative rates of evolution at pre- and postzygotic stages. Investigations of premating isolation are also necessary to understand the contribution reinforcement

makes to the evolution of reproductive isolation in plants. Fortunately, field-based studies of premating isolation are particularly tractable in plant species (see, for example, Schemske and Bradshaw 1999; Bradshaw and Schemske 2003), as are matching analyses of postpollination prezygotic isolation stages, including pollen-stigma, pollen-style, and pollen competitive interactions (Levin 1978; Arnold 1997). Similarly, more comprehensive direct comparisons of the patterns of reproductive isolation within and between diploid and polyploid species will provide a more precise account of the range of effects of genome doubling on the expression of reproductive isolation and its relationship with genic divergence. Finally, our findings suggest that while the historical (Stebbins 1950; Grant 1981) and recent (Rieseberg 1995; Arnold 1997; Soltis and Soltis 1999) emphasis on the role of hybridization, reticulation, and polyploidy in plant speciation processes is justified, additional approaches—including analyses developed to investigate speciation processes in animals—may provide complementary insights into common speciation patterns and processes in plants.

ACKNOWLEDGMENTS

We thank J. Antonovics, D. R. Taylor, M. Turelli, J. H. Willis, D. Baum, two anonymous reviewers, and members of the Willis and Turelli lab groups, for useful comments and suggestions. We also thank M. Mayer (University of San Diego) for kindly providing unpublished genetic distance data for *Streptanthus*, and T. Case, N. Tsutsui, and J. Hille Ris Lambers for contributing to the code used for permutation tests. LCM was supported in part by a James B. Duke Fellowship, and a University of California Davis Center for Population Biology Postdoctoral Fellowship. PT was supported in part by a National Research Initiative Competitive Grants Program, U.S. Department of Agriculture Award (99-353018076). MSO was supported in part by a National Science Foundation (NSF)/Alfred P. Sloan Postdoctoral Fellowship and NSF grant DEB-0317115.

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Corresponding Editor: D. Baum