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FLORAL MORPHOMETRICS AND THE EVOLUTION OF SEXUAL DIMORPHISM IN *LYCIUM* (SOLANACEAE)

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Abstract.—Plants of *Lycium californicum*, *L. exsertum*, and *L. fremontii* produce flowers that are either male-sterile (female) or hermaphroditic, and populations are morphologically gynodioecious. As is commonly found in gynodioecious species, flowers on female plants are smaller than those on hermaphrodites for a number of floral traits. Floral size dimorphism has often been hypothesized to be the result of either a reduction in female flower size that allows reallocation to greater fruit and seed production, or an increase in hermaphroditic flower size due to the increased importance of pollinator attraction and pollen export for hermaphroditic flowers. We provide a test of these two alternatives by measuring 11 floral characters in eight species of *Lycium* and using a phylogeny to reconstruct the floral size shifts associated with the evolution of gender dimorphism. Our analyses suggest that female flowers are reduced in size relative to the ancestral condition, whereas flowers on hermaphrodites have changed only slightly in size. Female and hermaphroditic flowers have also diverged both from one another and from ancestral cosexual species in several shape characteristics. We expected sexual dimorphism to be similar among the three dimorphic taxa, as gender dimorphism evolved only a single time in the ancestor of the American dimorphic lineage. While the floral sexual dimorphism is broadly similar among the three dimorphic species, there are some species-specific differences. For example, *L. exsertum* has the greatest floral size dimorphism, whereas *L. fremontii* had the greatest size-independent dimorphism in pistil characters. To determine the degree to which phylogenetic uncertainty affected reconstruction of ancestral character states, we performed a sensitivity analysis by reconstructing ancestral character states on alternative topologies. We argue that investigations such as this one, that examine floral evolution from an explicitly phylogenetic perspective, provide new insights into the study of the evolution of floral sexual dimorphism.

Key words.—Floral evolution, gynodioecy, *Lycium*, male-sterility, phylogeny, sensitivity analysis, sexual dimorphism, Solanaceae.

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In the majority of angiosperm species, individual plants make both male and female gametophytes and are cosexual in gender function. This is in sharp contrast to the pattern seen in higher animals in which male and female gametes are typically segregated among individuals and populations are usually dimorphic in gender expression. Though the number of plant species with dimorphic breeding strategies is low on a percentage basis (about 4–6% worldwide; Yampolsky and Yampolsky 1922; Renner and Ricklefs 1995), gender dimorphism is evolutionarily interesting because it has emerged repeatedly in many unrelated angiosperm lineages (Thomson and Brunet 1990; Geber et al. 1999).

The evolution of gender dimorphism in plants is often accompanied by changes in the floral structures of the sexual mating types. Indeed, this floral specialization has provided the opportunity for researchers to study the morphological divergence of females and hermaphrodites in gynodioecious populations and females and males in dioecious populations (e.g., Darwin 1877; Baker 1948; Lloyd and Webb 1977; Delph 1996; Delph et al. 1996; Eckhart 1999; Ashman 1999, 2000). For animal-pollinated species, it is unclear why flowers on females and hermaphrodites in gynodioecious populations should diverge in form since successful reproduction requires pollinators to visit both mating types (Charlesworth 1993; but see Willson and Ågren 1989). However, differences in optimal resource investment may result in the divergence of floral phenotypes that facilitate female or male function (Bateman 1948; reviewed in Delph 1996; Eckhart 1999). For instance, in gynodioecious populations, flowers on female plants are almost always smaller than flowers on hermaphroditic plants (Baker 1948; see Tables 8.1, 8.2 in Delph 1996; see Table 2 in Eckhart 1999). This observation has suggested to some researchers that resource savings associated with the reduction in flower size allow females to reallocate to fruit and seed production and, thus, aid their maintenance in gynodioecious populations (Eckhart 1992; Ashman 1994). Others have noted that if male fitness is limited by access to females, larger hermaphroditic flowers may be favored to increase visitation by pollinators and improve the male function of hermaphrodites (Bell 1985). The suggestion that larger corollas attract more pollinators has much empirical support (Galen and Newport 1987; Ashman and Stanton 1991; Vaughton and Ramsey 1998), and some studies have also found that pollinator service affects male fitness more strongly than female fitness (Bell 1985; Galen and Stanton 1989; Young and Stanton 1990; Vaughton and Ramsey 1998; but see Johnson et al. 1995). These two mechanisms (reduction in female flower size with subsequent compensation and increased hermaphroditic flower size to attract pollinators) are not mutually exclusive and both may affect flower size in gynodioecious populations.

Though comparisons of the sexual mating types within dioecious and gynodioecious populations may suggest adaptive explanations for divergence, they tell us little about how sexually dimorphic characters evolved in the first place. Comparisons with cosexual sister taxa should be more useful for understanding how females and hermaphrodites in gynodioecious populations evolve from ancestral cosexual populations (cf. Charlesworth 1993; Eckhart 1999). Phylogenetic comparisons can potentially tell us how the mating types of dimorphic species compare to their cosexual relatives, and

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TABLE 1. Locations of populations in southeastern Arizona for the dimorphic and cosexual species of *Lycium* included in this study.

Species	Population	Location
Dimorphic species		
L. californicum Nutt. ex Gray	Houser Road	32°46′48″N 111°37′48″W
L. exsertum A. Gray	Desert Peak	32°36′00″N 111°15′00″W
L. fremontii A. Gray	Casa Grande	32°47′06″N 111°42′22″W
	Houser Road	32°46′48″N 111°37′48″W
Cosexual species		
L. andersonii A. Gray	Desert Peak	32°36′00″N 111°15′00″W
	Organ Pipe	32°32′28″N 110°42′44″W
	Houser Road	32°46′44″N 111°37′23″W
L. berlandieri Dunal	Casa Grande	32°47′31″N 111°41′56″W
L. pallidum Miers	Oracle	32°32′28″N 110°42′44″W
L. parishii A. Gray	Organ Pipe	31°54′00″N 112°51′00″W
L. macrodon A. Gray	Desert Peak	32°36′00″N 111°15′00″W

how the sexual mating types have changed over evolutionary time.

In separate papers on the genus *Lycium* we have focused on the distribution of incompatibility systems and fruit and seed production (Miller and Venable 2002), the evolutionary dynamics of the origin of gynodioecy (Miller and Venable 2000), and the phylogeny of *Lycium* (Miller 2002). In this paper we compare the floral morphology of females and hermaphrodites in the three North American dimorphic species to five perfect-flowered, cosexual *Lycium* species. Using a phylogenetic framework (Miller 2002), we reconstruct the historical shifts in floral size and form across the transition from hermaphroditism to gynodioecy. Specifically, we ask whether the evolution of male-sterility in *Lycium* was accompanied by a reduction in flower size for females, an increase in flower size in hermaphrodites, or both. An increase in flower size in the hermaphrodites of the dimorphic species from their cosexual ancestor is consistent with the improved male function hypothesis for hermaphrodites, whereas a decrease in flower size for females is consistent with resource reallocation arguments. Further, we compare the pattern and degree of sexual dimorphism in floral characters among the three gynodioecious species. Gender dimorphism is hypothesized to have arisen one time in the ancestor of the American dimorphic species (Miller 2002); thus, we might expect sexual dimorphism to be similar across the three dimorphic species. Alternatively, if species had evolved to different points on the transition to dioecy, we might expect correlated differences in the degree of sexual dimorphism.

MATERIALS AND METHODS

Study Species

Lycium (Solanaceae) is a genus of approximately 75 shrubby perennials that are distributed worldwide in arid to semiarid environments, especially in southwestern North America, South America, and southern Africa (Hitchcock 1932; Joubert 1981; Bernardello 1986). Gender dimorphism is rare in Solanaceae, known in only six of 94 genera and in only about 0.8% of its species (Sawyer and Anderson 2000). Most *Lycium* species have perfect flowers on all plants and are cosexual in gender expression (Hitchcock 1932; Chiang-Cabrera 1981; Bernardello 1986). However, six of the 17 species occurring in Africa have been described as functionally dioecious (Minne et al. 1994; Venter et al. 1999). None of the 30 South American species are dimorphic in gender expression (Bernardello 1986). Previous studies of the North American members of the genus suggested that three of the North American species have gender dimorphism: *L. exsertum* and *L. fremontii* (Chiang-Cabrera 1981; Gilmartin 1983) and *L. californicum* (Miller and Venable 2002).

Variation in Floral Form

Flowers from plants existing in natural populations of eight species (three dimorphic and five cosexual) growing in southeastern Arizona were collected and preserved in formalin-acetic acid-alcohol (recipe from Kearns and Inouye 1993; Table 1). Recently opened flowers (<1 day old) from females and hermaphrodites of all dimorphic species were collected during March 1998 and 1999. Collections from the cosexual species were made during March and April 1998 for *L. andersonii*, *L. macrodon*, *L. pallidum*, and *L. parishii* and during September 1997 for *L. berlandieri*. Plants of the *Lycium* species studied here produce flowers in fascicles (typically 2–6 flowers); no more than one flower per fascicle was collected for this study. Two flowers on each plant from 54 plants (27 females and 27 hermaphrodites) were collected for each of the dimorphic species, with the exception of females for *L. fremontii*, in which only 25 plants were sampled. Two flowers from 27 plants of cosexual *L. berlandieri* and *L. parishii* and 12 plants of *L. andersonii*, *L. macrodon*, and *L. pallidum* were collected. There were no significant differences in the overall size (principal component 1; see Table 2 in Results) of flowers collected from different sites or in different seasons (Table 1; *L. andersonii*: $F_{2,9} = 0.736$, $P = 0.506$; *L. californicum*: $F_{1,50} = 3.68$, $P = 0.061$; *L. exsertum*: $F_{1,50} = 1.11$, $P = 0.298$; *L. fremontii*: $F_{1,48} = 1.78$, $P = 0.189$).

Flowers were dissected and floral structures measured under a dissecting microscope equipped with an ocular micrometer. For each flower, eleven measurements were made: calyx width, calyx tube length, calyx lobe length, corolla tube length, corolla width, distance of filament adnation along the corolla tube, length of the free portion of the filament, anther

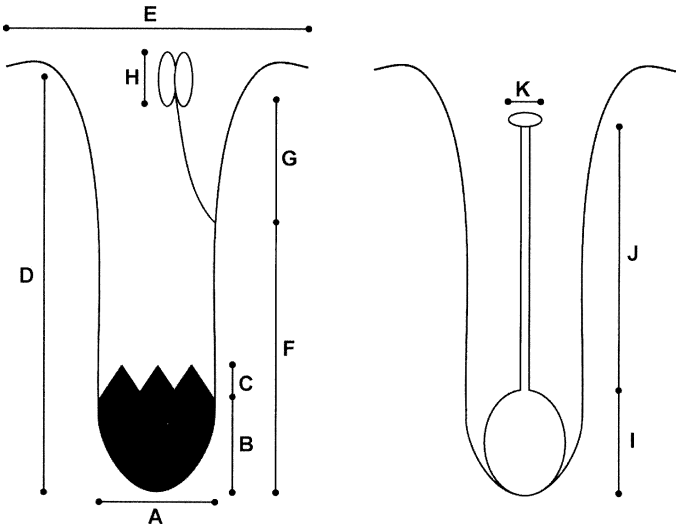

FIG. 1. Floral characters measured on species of *Lycium*. (A) calyx width, (B) calyx tube length, (C) calyx lobe length, (D) corolla tube length, (E) corolla width, (F) adnation of filament along the corolla, (G) filament length, (H) anther length, (I) ovary length, (J) style length, and (K) stigma width.

length, ovary length, style length, and stigma width (Fig. 1). Although flowers on female plants of the dimorphic species do not produce pollen, they do possess rudimentary filaments and anthers, which were measured as in dimorphic hermaphrodites and cosexual species.

Principal component analysis (PROC PRINCOMP, SAS Institute 1989) was used to explore and summarize variation in the measured floral characters for all species. The two replicate flowers on plants were averaged and plant means were used for this analysis. All measurements were natural log transformed and standardized to have a mean of zero and a variance of one prior to the principal components analysis. We used a general linear model (PROC GLM, SAS Institute 1989) to test whether species-morph combinations differed in size and shape as defined by the first three principal components (PC1 = size dimension; PC2, PC3 = shape dimensions). A species-morph combination included both the species designation and also the sexual mating type (e.g., female, hermaphrodite, or cosexual) of the plant. The Ryan-Einot-Gabriel-Welsch (REGWQ, SAS Institute 1989) multiple comparison procedure was used to compare all pairwise species-morph combinations. In addition, we used planned contrasts to test for significant differences among the three dimorphic species in the degree of sexual dimorphism (i.e., species-by-gender interaction). Unless stated otherwise, significance in this paper means $P < 0.05$.

Data for each floral character were natural log transformed and analyzed using general linear models that included the fixed effect of species-morph combination and the random effect of plant nested within species-morph combination (PROC GLM, SAS Institute 1989). We used the REGWQ multiple comparison procedure to compare all pairwise species-morph combinations, and the contrast statement to test for a species-by-gender interaction among the dimorphic species.

Since there were overall floral size differences between species and genders, we conducted analyses of covariance (PROC GLM, SAS Institute 1989) of the fixed effect of species-morph combination, correcting for overall flower size (as indicated by PC1; see Table 2 in Results). This enabled us to test whether floral traits were large or small for a flower of a particular size. For example, stigmas in the larger hermaphroditic flowers might be as wide or wider than stigmas in the smaller female flowers, but still be narrower than in females after correcting for the difference in flower size (reflecting a shape shift in hermaphrodites that de-emphasizes stigmas). We used the contrast statement to test for a species-by-gender interaction for the three dimorphic taxa, and compared the size-adjusted least squares means between all species-morph combinations using the Tukey-Kramer multiple comparison procedure as implemented in SAS (SAS Institute 1989).

Phylogeny

A hypothesis of phylogenetic relationships for the species included in this study is redrawn here from the single most parsimonious tree of a combined analysis of molecular sequence (internal transcribed spacer regions of nuclear ribosomal DNA) and morphological data from a larger study of *Lycium* (Fig. 2; see also Miller 2002). Bootstrap values for the branching relationships were obtained from two hundred bootstrap searches (Felsenstein 1985), each with 10 random addition sequence replicates and TBR branch swapping. The three North American dimorphic species are well supported as monophyletic, strongly suggesting a single origin of gender dimorphism (Fig. 2). The sister-taxon relationships between *L. andersonii* and *L. berlandieri*, *L. macrodon* and *L. pallidum*, and dimorphic *L. exsertum* and *L. fremontii* are also well supported (Fig. 2).

We obtained estimates of branch lengths for the most parsimonious topology (Miller 2002) using maximum-likelihood (PAUP* ver. 4.0b10; Swofford 2002). The model of molecular evolution used for calculating branch lengths corresponds to that of Hasegawa et al. (1985), with among-site rate heterogeneity modeled as a discrete approximation to a gamma distribution with shape parameter alpha. Nucleotide frequencies were empirically determined and the transition to transversion ratio, proportion of invariable sites, and the gamma distribution shape parameter were estimated.

Evolution of Floral Form

To examine the evolution of floral size, shape, and gender we overlaid the phylogeny of *Lycium* (Fig. 2) onto principal component space (Fig. 5). Because the evolution of gender dimorphism appears to have occurred only one time in North America (see Fig. 2), we show the split between females and hermaphrodites once and map the subsequent evolution of the dimorphic lineage separately for females and hermaphrodites. Values for principal components 1–3 were inferred for ancestral species (i.e., nodes in the phylogeny) using generalized least squares as implemented in COMPARE (Martins 2001). Ancestral character states were calculated for the most parsimonious topology both using branch lengths as estimated by likelihood and with branch lengths assumed to be

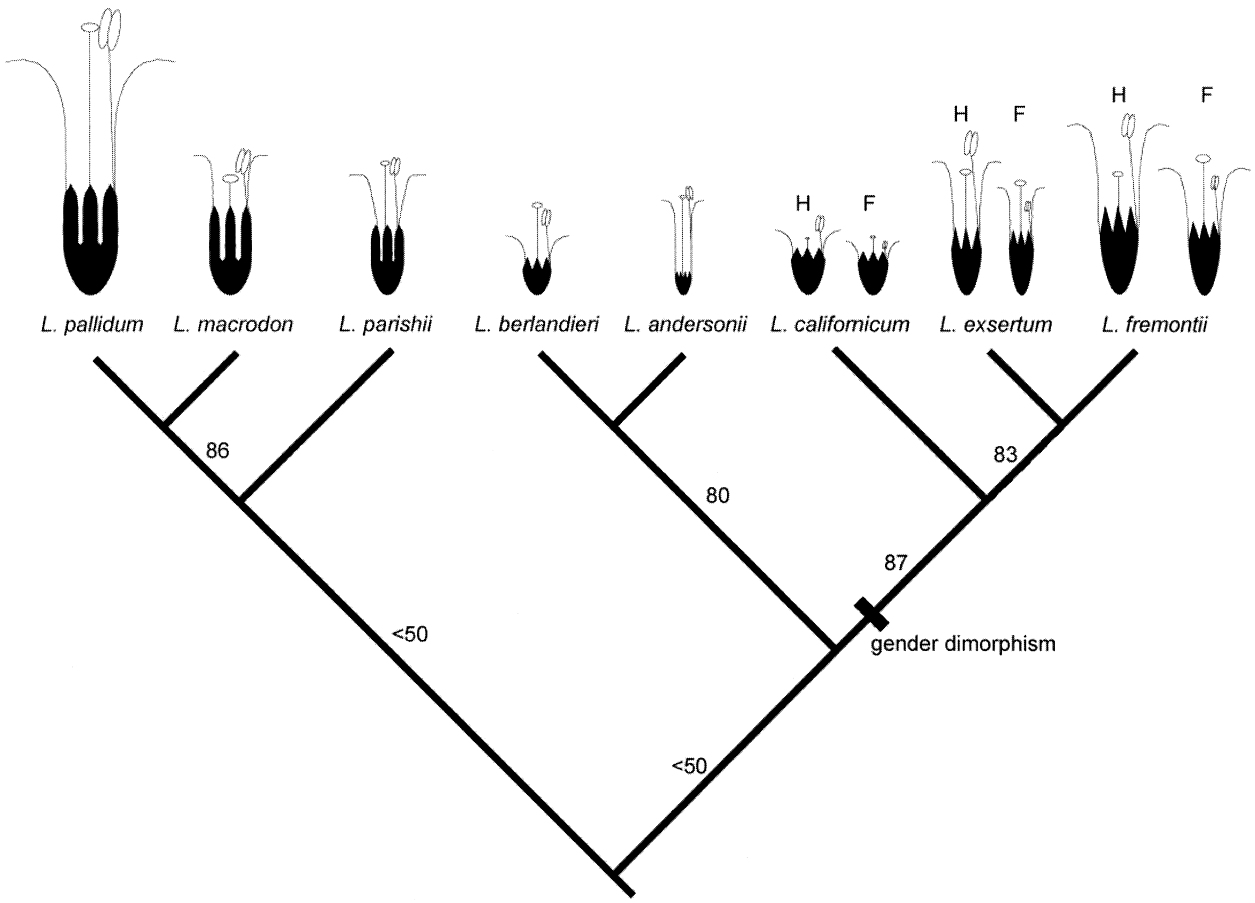

FIG. 2. Hypothesis of phylogenetic relationships for the species of *Lycium* included in this study. Details of the analysis based on molecular (internal transcribed spacer regions of nuclear ribosomal DNA) and morphological data are available in Miller (2002). Values on branches are bootstrap percentages, which indicate support for branching patterns. Flowers are drawn to scale relative to each other and represent the average flower dimensions measured for each species-morph combination. H, hermaphrodite; F, female.

equal. Ancestral state reconstructions were performed twice: ancestral states for females (i.e., nodes 2f and 3f in Fig. 5) were calculated using values of extant females, whereas ancestral states for hermaphrodites (i.e., nodes 2h and 3h in Fig. 5) were calculated using extant hermaphrodite scores. Since these analyses yielded two (similar) estimates of ancestral states for all other ancestral nodes, we averaged values from these two analyses for the other nodes in Figure 5.

Most phylogenetic reconstructions, including the one used here (Fig. 2), have some strongly supported and some weakly supported parts of the branching structure. In addition, inclusion of branch length information can often dramatically effect ancestral character state reconstructions (Martins 2001). In light of these uncertainties, we performed a sensitivity analysis to examine the robustness of our conclusions to alternative topologies and branch length assumptions (Donoghue and Ackerly 1996). Several nodes of the most parsimonious tree have low bootstrap support (bootstrap percentage < 50; Fig. 2). Therefore, we created all possible alternative topologies by altering the positions of weakly supported groups. We then reconstructed ancestral states for principal components 1–3 on these 15 alternative topologies, both incorporating branch length information and assuming equal branch lengths.

RESULTS

Variation in Floral Form

The first principal component (PC1) accounted for 63% of the variation in the dataset and was an index of overall flower size (Table 2). The eigenvector associated with PC1 had positive loadings for all the floral characters and these loadings were of similar magnitude, characteristic of a size vector. Large-flowered, cosexual *L. pallidum* and hermaphrodites of *L. fremontii* had high values for PC1, whereas the small-flowered species, *L. andersonii*, *L. berlandieri*, and *L. californicum* had low PC1 values (Figs. 2, 3A). All of the cosexual species were significantly different from each other, and hermaphrodites of the three dimorphic species were significantly larger than conspecific females (Figs. 2, 3A). Principal component 2 (PC2) accounted for 12% of the variation and described nonallometric variation in shape and gender-related traits (Table 2). Plants with high scores for PC2 tended to have wide calyces and corollas for flowers their size, but relatively short styles, narrow stigmas, and short adnation distances along the corolla tube. Species-morph combination explained a significant amount of the variation in PC2, and hermaphrodites of dimorphic species had significantly higher values for PC2 than conspecific females (Fig. 3B). The latter

TABLE 2. Character loadings and the proportion of variance explained for the first three principal components (PC) of the analysis for all eight *Lycium* species.

Floral character	PC1	PC2	PC3
Eigenvalue	6.91497	1.30349	0.83352
Proportion variance explained	0.628634	0.118499	0.075774
Eigenvectors:			
Calyx tube length	0.235062	0.159225	0.751810
Calyx width	0.238256	0.632869	0.048965
Calyx lobe length	0.303986	0.121117	−0.009879
Corolla tube length	0.354484	−0.170046	0.037411
Corolla width	0.309144	0.381495	−0.030298
Filament adnation	0.296516	−0.326247	−0.016444
Filament length	0.334490	0.035496	−0.285566
Anther length	0.297485	0.156286	−0.526923
Ovary length	0.337591	−0.125712	0.142306
Style length	0.310204	−0.353909	−0.109667
Stigma width	0.275403	−0.334400	0.195511

pattern is mostly a reflection of the reduction of female organs in hermaphrodites (Fig. 2). The third principal component (PC3) accounted for 8% of the variation and described some independent aspects of floral shape (calyx tube length) and gender (anther length). PC3 diverges significantly for females of dimorphic species compared to conspecific hermaphrodites and the cosexual species (Fig. 3C), reflecting the fact that female flowers had long calyces and short anthers for flowers their size (Fig. 2).

There was significant variation for all floral measurements, both among species (for all characters, 73–90% of the variation was due to species-morph combination) and for plants nested within species-morph combination (for all characters, 8–21% of the variation was due to plants nested within species-morph combination). In *L. pallidum* the corolla was 395% longer than in *L. berlandieri* and 374% wider than in *L. andersonii* (Table 3; Fig. 2). Floral measurements for the cosexual species of *Lycium* encompassed the range of values found in the dimorphic species except for a few characters on the small-flowered dimorphic *L. californicum* (Table 3). Comparison of hermaphrodites and females of dimorphic species reveals that flowers on hermaphroditic plants are significantly longer and wider for all calyx and corolla traits measured except for calyx tube length in *L. californicum* and *L. exsertum*, which is not different for the two genders (Table 3). As expected, females had reduced male sex organs: anthers and filaments on females were roughly half as long as those on hermaphrodites (Table 3). Despite the fact that flowers of hermaphrodites were larger than females for most characters measured, stigmas were wider in females of *L. californicum* and *L. fremontii* compared to conspecific hermaphrodites, and style length was longer for females compared to hermaphrodites in *L. fremontii* (Table 3). Surprisingly, ovaries were longer in hermaphrodites than females (except for *L. californicum*; Table 3).

For all calyx and corolla traits, the size-adjusted means are equivalent between females and hermaphrodites in the dimorphic species, except for calyx tube length in *L. exsertum*, which is longer for females (Table 4). Calyces are significantly longer in females of dimorphic species compared to the cosexual taxa (Table 4). For male sexual traits, anther length is significantly shorter in females of dimorphic species compared to both conspecific hermaphrodites and the cosexual species, and filaments are shorter in females compared to conspecific hermaphrodites (Table 4). Though differences in ovary size are not significantly different between females and hermaphrodites when adjusted for flower size, both style length and stigma width are larger in females compared to conspecific hermaphrodites (Table 4).

The two-way ANOVA of PC1 showed that there is a greater size dimorphism in *L. exsertum* than in the other two dimorphic species (significant species-by-gender interaction, $F_{2,239} = 5.41$, $P = 0.005$). Analyses of single traits confirmed significant differences among species in size dimorphism (i.e., interactions between species and gender) for the two floral width characters and two androecial traits (Fig. 4A). Species differences in sexual dimorphism for PC2 (size-independent variation in calyx and corolla widths, filament adnation, style length, and stigma width) were not significant in the two-way ANOVA (species-by-gender interaction, $F_{2,239} = 1.06$, $P = 0.3478$), though *L. fremontii* was slightly more dimorphic and *L. exsertum* slightly less than *L. californicum*. When traits were analyzed individually, *L. fremontii* had greatest sexual dimorphism in absolute style length and stigma width (Fig. 4A) and also in flower size-corrected style length and stigma width (Fig. 4B). There was greater sexual dimorphism in *L. exsertum* for PC3 (calyx and anther lengths) indicated by the significant species-by-gender interaction ($F_{2,239} = 4.64$, $P = 0.0106$) for the two-way ANOVA. This is reflected in greater flower size-corrected dimorphism of *L. exsertum* in these two traits individually (Fig. 4B).

Evolution of Floral Form

The evolution of cosexual species has mostly involved differentiation in overall flower size (PC1, Fig. 5A). In contrast, shape and gender-related traits have evolved little among the cosexual species, and there is little separation for these species in Figure 5B. Only *L. andersonii* and, to a lesser extent, *L. pallidum* have nonallometric differences in floral shapes (Fig. 5B). *Lycium andersonii* has evolved flowers that are quite narrow for *Lycium* (Fig. 2), and allocation to the calyx is reduced for a flower its size (note the displacement

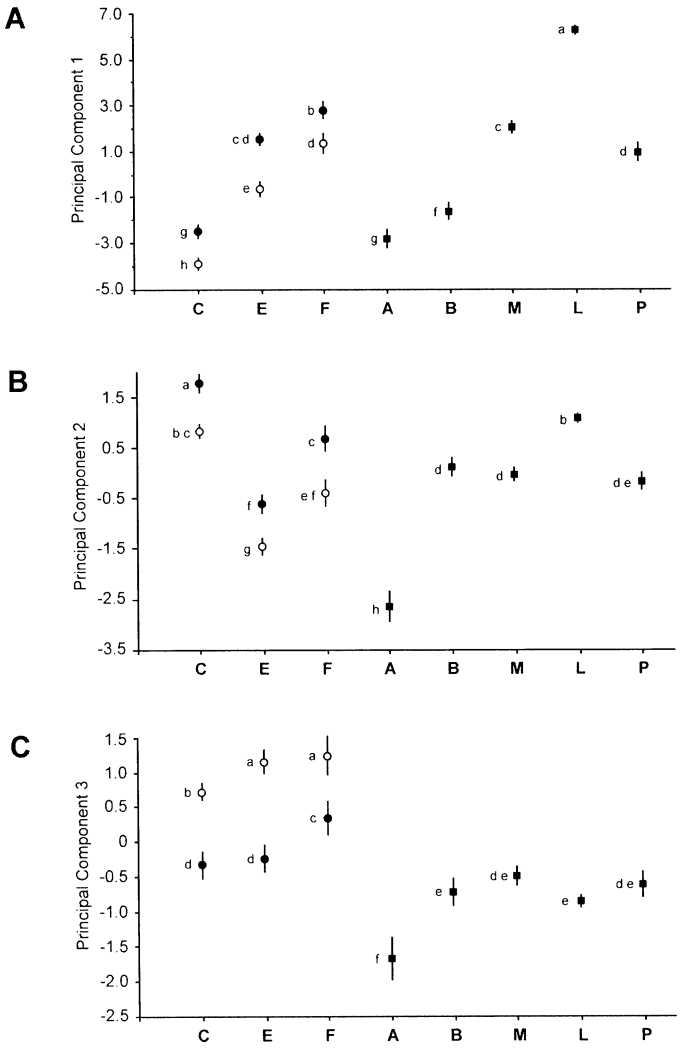

FIG. 3. Mean values ± 1 SD of (A) principal component 1, (B) principal component 2, and (C) principal component 3 for each species-morph combination. Significance ($P < 0.05$) as determined by the REGWQ multiple comparison procedure as implemented in SAS (SAS Institute 1989). Species-morph combinations sharing the same letter are not statistically different. C, *L. californicum*; E, *L. exsertum*; F, *L. fremontii*; A, *L. andersonii*; B, *L. berlandieri*; M, *L. macrodon*; L, *L. pallidum*; P, *L. parishii*. ○, females of dimorphic species; ●, hermaphrodites of dimorphic species; ■, cosexual species.

of *L. andersonii* along PC2 in Figs. 5A, B). By contrast, *L. pallidum* has evolved broad and flaring flowers (Fig. 2) with a wide calyx and long, free filaments (note the shift along PC2 for *L. pallidum* in Figs. 5A, B). The other three cosexual species are not significantly different from each other with regard to the PC2 shape component (Fig. 3B) and have values similar to that near the root of the tree (Fig. 5B). Compared to PC2, PC3 (relatively long calyces and short anthers) varies considerably less among the cosexual species, although it decreased for *L. andersonii* when it split from *L. berlandieri* (Fig. 5B).

The origin of the dimorphic lineage, as indicated by the dashed lines in Figure 5, was accompanied by a reduction in the size (PC1) of female flowers with little change in the size of hermaphroditic flowers (Fig. 5A). Subsequently there were increases (*L. fremontii* and *L. exsertum*) and decreases (*L. californicum*) in floral size of the dimorphic species, while conserving the sexual size dimorphism in each species. The phylogenetic reconstruction also suggests that both female and hermaphroditic flowers diverged in shape from their nearest common ancestor with cosexual species, though in different ways (Fig. 5B). Flowers on hermaphrodites diverged mostly by increasing PC2 (a correlated suite of traits involving widening of flowers and diminution of female structures), whereas flowers on females diverged mainly by increasing PC3 (i.e., increasing calyx tube length and decreasing anther length). Subsequent changes in the shape of flowers on hermaphroditic plants involve both increases and decreases in PC2, with little change in PC3. Evolution of the shape of flowers on female plants also mostly involves changes in PC2. PC3 for flowers on female plants remains consistently higher than for flowers on hermaphroditic plants or flowers on the cosexual species (Figs. 3C, 5B).

All alternative reconstructions (Fig. 6A) showed a reduction in flower size (PC1) for flowers on ancestral females (i.e., node 2f in Fig. 5) and little change in flower size on ancestral hermaphroditic plants (i.e., node 2h in Fig. 5). Similarly, all reconstructions showed an increase in PC2 for flowers on hermaphrodites, whereas females changed little in this shape component (Fig. 6B). The reverse pattern was true for PC3: females increased substantially under all topologies, whereas flowers on hermaphrodites remained similar in this component to their most recent common cosexual ancestor (Fig. 6C).

DISCUSSION

Reduction of Female Flowers

Flowers on female plants are significantly smaller than those on conspecific hermaphrodites (Table 3; Fig. 3A), as is the case in most gynodioecious populations across a wide variety of plant families (e.g., Delph 1990; Gibson and Diggle 1997; Puterbaugh et al. 1997; Humeau et al. 1999; Williams et al. 2000; reviewed in Baker 1948; Delph 1996; Eckhart 1999). Our phylogenetic analysis suggests that the transition to gender dimorphism was accompanied by a reduction of female flower size, with little change in hermaphroditic flower size (Fig. 5A, dashed lines). This pattern is more consistent with the resource reallocation hypothesis than the improved male function hypothesis as an explanation for the original transition to gender dimorphism. This is because the improved male function hypothesis predicts that flowers on hermaphrodites (in gynodioecious species) become larger to attract pollinators for increased mating opportunities, a pattern not supported by our data. This result is reasonable since sexual selection on males is not widely accepted as a force favoring the separation of gender functions (Lloyd 1982).

The apparent reduction of flower size on female plants could be due to increased fitness available through reallocation to additional seeds, fruits, or flowers on female plants. Analyses of resource reallocation indicate that, despite substantial resource savings in females due to the loss of male function (nearly 20% of total floral biomass), females of dimorphic *Lycium* species do not respond by making increased numbers of seeds per fruit, fruits per flower, or flowers per

TABLE 3. Means (± 1 SD) for each floral character (see Fig. 1) for five cosexual and three dimorphic species of *Lycium* as calculated from plant means (PROC MEANS, SAS Institute 1989). Differences between species-morph combination (for all characters, $F_{10,239} \geq 84.53$, $P < 0.0001$) and plants nested within species-morph combination (for all characters, $F_{239,250} \geq 1.96$, $P < 0.0001$) were tested by separate general linear models for each character. For each floral character, means sharing the same superscript do not differ significantly. C, cosexual; F, female; H, hermaphrodite. Units are in millimeters.

Floral character	*L. andersonii* C	*L. berlandieri* C	*L. macrodon* C	*L. pallidum* C	*L. parishii* C	*L. californicum* F	*L. californicum* H
Calyx tube length	1.39 (±0.27)^{g}	2.03 (±0.33)^{f}	2.59 (±0.42)^{e}	3.95 (±0.41)^{c}	2.62 (±0.39)^{e}	2.56 (±0.31)^{e}	2.80 (±0.45)^{e}
Calyx width	1.26 (±0.22)^{f}	2.23 (±0.20)^{d}	3.02 (±0.18)^{b}	4.30 (±0.26)^{a}	2.53 (±0.25)^{c}	2.32 (±0.16)^{d}	2.61 (±0.19)^{c}
Calyx lobe length	0.48 (±0.16)^{g}	0.95 (±0.33)^{ef}	4.55 (±0.97)^{a}	4.53 (±0.38)^{a}	2.83 (±0.42)^{b}	0.82 (±0.15)^{f}	0.97 (±0.15)^{e}
Corolla tube length	7.50 (±0.88)^{g}	4.69 (±0.63)^{i}	10.96 (±1.73)^{c}	18.53 (±0.89)^{a}	9.50 (±1.56)^{e}	4.19 (±0.59)^{j}	5.08 (±0.68)^{h}
Corolla width	3.57 (±0.50)^{h}	5.06 (±0.69)^{e}	6.17 (±0.55)^{d}	13.36 (±1.03)^{a}	6.02 (±0.90)^{d}	4.26 (±0.45)^{f}	5.21 (±0.57)^{e}
Filament adnation	4.45 (±0.65)^{c}	1.75 (±0.34)^{g}	5.77 (±1.16)^{a}	5.08 (±0.57)^{b}	4.54 (±0.93)^{c}	1.79 (±0.24)^{fg}	1.95 (±0.26)^{f}
Filament length	3.82 (±0.48)^{g}	4.38 (±0.67)^{f}	4.21 (±0.56)^{f}	16.27 (±1.49)^{a}	5.77 (±1.00)^{d}	2.15 (±0.31)^{h}	3.67 (±0.54)^{g}
Anther length	1.07 (±0.13)^{e}	1.30 (±0.19)^{cd}	1.94 (±0.18)^{b}	3.19 (±0.25)^{a}	1.38 (±0.21)^{c}	0.61 (±0.08)^{g}	1.27 (±0.19)^{d}
Ovary length	1.25 (±0.34)^{f}	1.65 (±0.19)^{e}	1.87 (±0.16)^{d}	3.30 (±0.33)^{a}	1.58 (±0.26)^{e}	1.03 (±0.10)^{g}	1.07 (±0.11)^{g}
Style length	6.37 (±1.16)^{e}	5.22 (±0.87)^{f}	6.98 (±0.96)^{cd}	17.44 (±1.12)^{a}	8.60 (±1.56)^{b}	3.34 (±0.38)^{g}	3.27 (±0.72)^{g}
Stigma width	0.48 (±0.11)^{f}	0.80 (±0.16)^{cd}	1.20 (±0.09)^{b}	1.37 (±0.20)^{a}	0.66 (±0.14)^{e}	0.38 (±0.06)^{g}	0.30 (±0.06)^{h}

branch compared to their cosexual relatives (Miller 2000). Also, females do not allocate additional biomass to larger gynoecia within flowers compared to their cosexual relatives (Miller 2000). Although reallocation of resources by females could still operate by mechanisms not yet studied (e.g., longer lifespan, maternal effects on offspring quality), to date there is not strong evidence for reallocation by females of dimorphic *Lycium* species.

Alternatively, floral size reduction could be the result of a developmental association between flower size and anther production (Weiss and Halevy 1989). For example, Weiss and Halevy (1989) demonstrated that removal of anthers inhibited corolla growth in *Petunia* (Solanaceae) and that gibberellin augmentation partially substituted for the excised stamens. They hypothesized that gibberellin produced in the anthers was transported to the corolla where it stimulated corolla growth. We have observed the presence of an apparent somatic mutation to male-sterility on an otherwise hermaphroditic plant of dimorphic *L. fremontii*. On this plant, all flowers were hermaphroditic with the exception of the distal portion (about 30 cm) of one branch, where flowers were male-sterile. Interestingly, there was a noticeable size dimorphism between the hermaphroditic and male-sterile flowers on this plant, suggesting that the reduction in flower size on female plants may be associated developmentally with male sterility (see also Plack 1957, 1958; Widén and Widén 1999). However, in a literature survey designed to test the generality of the developmental correlation hypothesis, Delph et al. (1996) found no consistent pattern of increased corolla size in staminate flowers across many monoecious and dioecious species and, thus, no support that this hypothesis is broadly applicable. Flower emasculations could be used to experimentally test the developmental correlation hypothesis for *Lycium* (cf. Plack 1957, 1958).

Sensitivity Analysis

The reconstruction of floral size change presented in Figure 5 is based on the most parsimonious topology and incorporates estimates of branch lengths. Our finding, that sexual dimorphism in *Lycium* was accompanied by a reduction of flower size in females with little change for hermaphrodites, is robust to all alternative topologies, and also to reconstructions assuming equal branch lengths (Fig. 6A). This provides strong support for our conclusion regarding flower size changes associated with the evolution of gender dimorphism in *Lycium*.

A further source of phylogenetic uncertainty is that the five cosexual species included in this study are only a subset of approximately 75 species of *Lycium* found throughout the world. Thus, the true sister taxon to the dimorphic clade might not be drawn from the species we measured. Three larger analyses of *Lycium* involved an internal transcribed spacer (ITS) molecular sequence dataset for 25 species from five continents, a combined ITS and morphological dataset for 18 species (Miller 2002), and an independent chloroplast sequence dataset for 23 species (Fukuda et al. 2001). In all of these analyses the well-supported *L. andersonii* plus *L. berlandieri* group comes out as the sister group to the dimorphic species, but with low bootstrap support. In the 25-species dataset of DNA sequences, there are 393 trees that are a single step longer than the most parsimonious trees. Eighty percent of these trees have one of the following clades as sister taxa to the dimorphic species: *L. andersonii* + *L. berlandieri*, *L. carolinianum* + *L. sandwicense*, or *L. parishii* + *L. torreyi* + *L. brevipes*. In the analysis of the 18-species dataset combining molecular and morphological data, there are 261 trees that are up to three steps longer than the most parsimonious tree. More than 80% of these trees have either *L. andersonii* + *L. berlandieri* or *L. carolinianum* + *L. sandwicense* as sister to the dimorphic species. We do not have the original data to examine longer trees in the Fukuda et al. (2001) analysis. However, it seems likely from these three analyses, one of which involves a dataset totally independent from the other two, that the *L. andersonii* + *L. berlandieri* clade is the sister taxon to the dimorphic species. If not, then it is likely that the sister taxon is either *L. parishii* or the clade with *L. carolinianum* + *L. sandwicense*. Judging from our own (*L. parishii*) and previously published flower measurements (*L. carolinianum* and *L. sandwicense*; Hitchcock 1932; Chiang-Cabrera 1981), all of these sister configurations involve flowers that are as large as or larger than *L. andersonii* and *L. berlandieri*. Because of this, reconstruction of the transition to gynodioecy involving any of these groups would

TABLE 3. Extended.

L. exsertum		*L. fremontii*	
F	H	F	H
3.66 ($\pm$0.63)^{d}	3.67 ($\pm$0.80)^{d}	4.33 ($\pm$0.69)^{b}	4.74 ($\pm$0.92)^{a}
1.86 ($\pm$0.23)^{e}	2.32 ($\pm$0.19)^{d}	2.51 ($\pm$0.34)^{c}	3.01 ($\pm$0.27)^{b}
1.46 ($\pm$0.37)^{d}	1.70 ($\pm$0.42)^{c}	1.45 ($\pm$0.28)^{d}	1.95 ($\pm$0.56)^{c}
8.19 ($\pm$0.84)^{f}	10.66 ($\pm$1.32)^{c}	10.02 ($\pm$1.19)^{d}	13.37 ($\pm$1.53)^{b}
3.90 ($\pm$0.42)^{g}	5.20 ($\pm$0.46)^{e}	7.19 ($\pm$0.98)^{c}	8.27 ($\pm$0.87)^{b}
3.43 ($\pm$0.52)^{e}	4.78 ($\pm$0.71)^{bc}	4.09 ($\pm$1.11)^{d}	5.24 ($\pm$1.05)^{b}
3.83 ($\pm$0.54)^{g}	7.44 ($\pm$1.08)^{c}	4.79 ($\pm$1.04)^{e}	8.92 ($\pm$0.99)^{b}
0.82 ($\pm$0.10)^{f}	1.94 ($\pm$0.18)^{b}	1.08 ($\pm$0.26)^{e}	1.87 ($\pm$0.24)^{b}
1.83 ($\pm$0.21)^{d}	2.03 ($\pm$0.22)^{c}	2.15 ($\pm$0.20)^{c}	2.44 ($\pm$0.22)^{b}
6.82 ($\pm$0.80)^{cde}	7.43 ($\pm$1.13)^{c}	8.25 ($\pm$1.50)^{b}	6.78 ($\pm$1.88)^{de}
0.87 ($\pm$0.18)^{c}	0.86 ($\pm$0.19)^{c}	1.14 ($\pm$0.19)^{b}	0.79 ($\pm$0.27)^{d}

conserve our conclusion, that the origin of gynodioecy involved a reduction in female flower size with little to no change in flower size in hermaphrodites. Thus, while the phylogeny is not known with complete certainty, the conclusions concerning the evolution of flower size appear to be robust to this uncertainty.

The Evolution of Floral Shape and Gender-Related Traits

The reconstruction in Figure 5 suggests that the transition to gender dimorphism was accompanied by an increase in the PC2 shape component for flowers on ancestral hermaphrodites, with virtually no change in this shape component for flowers on ancestral females. Further, this result is consistent across all possible alternative resolutions for weakly supported nodes in Figure 2 (Fig. 6B). The increase in PC2 for hermaphrodites coincident with the origin or separate sexes is mostly due to a reduction, relative to flower size, in style length and stigma width, with little or no change in the floral width traits contributing to PC2 (Fig. 2). This suggests that some reduction of female function in hermaphrodites may have occurred coincident with the evolution of gender dimorphism or at least prior to the diversification of the dimorphic species. The morphological diminution of female reproductive organs in hermaphrodites is common among species evolving dioecy via the gynodioecious pathway. However, it is not known how soon following the origin of gender dimorphism these morphological changes emerge (Geber et al. 1999). Support for the idea of early reduction of female organs in *Lycium* comes from the observation that the sexual dimorphism in the three dimorphic species is similar (note the relative positions in PC space of females and hermaphrodites within each of the dimorphic species [Figs. 5A, B]). This suggests a common origin of morphological change in these structures prior to speciation in the dimorphic lineage.

In addition to describing differences in gynoecia between genders within the dimorphic species, PC2 also describes shape variation among species. Flowers of *L. andersonii* have a distinctive shape with a calyx and corolla that are considerably narrower relative to flower size compared to other species, but with long styles and well-developed stigmas for

TABLE 4. Least-squares means for each floral character (see Fig. 1) for five cosexual and three dimorphic species of *Lycium* as calculated from separate general linear models for each character using size (principal component 1) as a covariate (SAS Institute 1989). Values are back-transformed means. For each floral character, means sharing the same superscript do not differ significantly. C, cosexual; F, female; H, hermaphrodite. Units are in millimeters.

	L. andersonii	*L. berlandieri*	*L. macrodon*	*L. pallidum*	*L. parishii*	*L. californicum*		*L. exsertum*		*L. fremontii*	
Floral character	C	C	C	C	C	F	H	F	H	F	H
Calyx tube length	2.03^{ab}	2.53^{gh}	1.93^{ab}	1.64^{a}	2.28^{bh}	4.42^{e}	3.96^{ef}	3.96^{e}	2.91^{dg}	3.56^{ce}	3.15^{cdfg}
Calyx width	1.58^{f}	2.56^{e}	2.55^{ae}	2.54^{abde}	2.33^{abe}	3.23^{d}	3.24^{d}	1.96^{c}	2.04^{c}	2.23^{b}	2.37^{abe}
Calyx lobe length	0.73^{f}	1.20^{e}	3.18^{b}	1.59^{acde}	2.40^{a}	1.55^{cd}	1.47^{cd}	1.58^{d}	1.28^{cde}	1.14^{ce}	1.18^{ce}
Corolla tube length	11.35^{f}	5.94^{e}	8.06^{abcd}	7.39^{adeg}	8.19^{abcd}	7.41^{bd}	7.34^{d}	8.98^{cg}	8.48^{abc}	8.20^{abcd}	8.81^{bc}
Corolla width	4.73^{bg}	5.94^{ef}	5.02^{b}	7.07^{af}	5.43^{be}	6.31^{ade}	6.71^{ad}	4.14^{c}	4.45^{cg}	6.23^{af}	6.20^{af}
Filament adnation	6.80^{g}	2.21^{f}	4.16^{b}	1.95^{af}	3.87^{bc}	3.24^{abcde}	2.86^{e}	3.75^{bd}	3.76^{bc}	3.23^{de}	3.36^{cde}
Filament length	5.26^{abf}	5.24^{fij}	3.32^{ch}	7.91^{a}	5.13^{bf}	3.34^{gh}	4.86^{ef}	4.09^{d}	6.20^{ai}	4.00^{dfeg}	6.44^{aij}
Anther length	1.49^{abd}	1.57^{bd}	1.51^{bd}	1.50^{abd}	1.22^{a}	0.97^{c}	1.71^{d}	0.88^{c}	1.61^{d}	0.89^{c}	1.33^{ab}
Ovary length	1.61^{efg}	1.93^{c}	1.53^{abg}	1.77^{acf}	1.42^{bg}	1.51^{abde}	1.37^{ab}	1.94^{c}	1.74^{cde}	1.88^{cf}	1.85^{cde}
Style length	9.22^{f}	6.44^{acd}	5.28^{bd}	7.50^{acf}	7.48^{af}	5.64^{cde}	4.51^{b}	7.40^{a}	6.00^{cd}	6.80^{ac}	4.48^{be}
Stigma width	0.72^{ace}	1.01^{b}	0.88^{be}	0.52^{acd}	0.57^{ac}	0.68^{ace}	0.43^{d}	0.94^{b}	0.67^{c}	0.92^{be}	0.49^{ad}

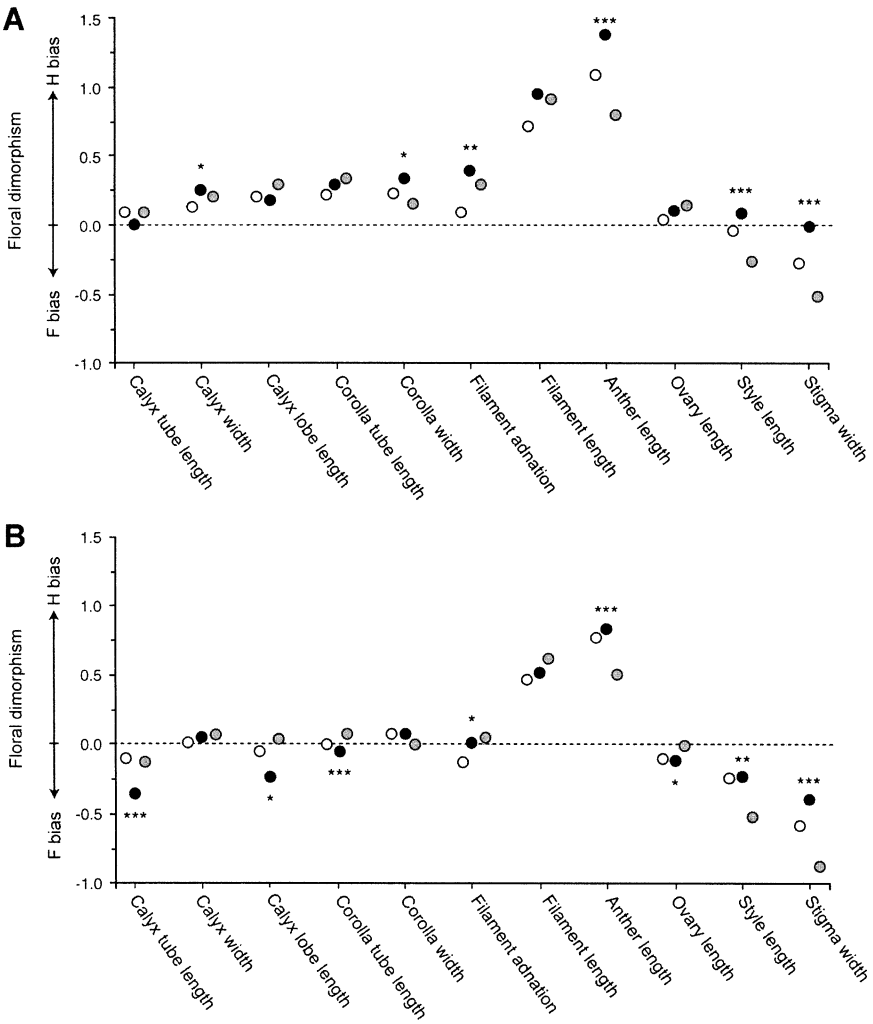

FIG. 4. Floral sexual dimorphism in the (A) original and (B) size-corrected floral measurements for dimorphic *Lycium californicum* (open circles), *L. exsertum* (closed circles), and *L. fremontii* (shaded circles). Floral size-corrected measurements are least-squares means from analyses of covariance using floral size (PC1) as a covariate. Sexual bias was determined for each character by dividing the mean value for the larger gender by the smaller gender and subtracting one, giving the proportional sexual bias. Traits for which hermaphrodites were larger than females were made positive, whereas traits for which females were larger than hermaphrodites were made negative. Significant differences in the degree of sexual dimorphism among species (i.e., species by gender interaction) for each floral character are indicated by $*P < 0.05$, $**P < 0.005$, and $***P < 0.001$.

flowers their size (Fig. 2). In addition, following the evolution of gender dimorphism, changes in the PC2 shape component involved decreases for both genders in *L. exsertum* and increases for both genders in *L. californicum* (Fig. 5B). This is reflected in the fact that flowers for both genders in *L. exsertum* are relatively narrow and have long styles and large stigmas for flowers their size, whereas those for *L. californicum* are wide with shorter styles and smaller stigmas for flowers their size (Fig. 2). It would be interesting to know whether any of these shape differences match differences in pollination, for example a pollinator shift for *L andersonii*. The limited data on pollination suggest that these species are pollinated by anthophorid long-tongued bees, although smaller bees and hummingbirds have also been observed visiting *Lycium* (J. S. Miller, unpubl. obs.). However, there is little quantitative data on visitation rates or pollinator effectiveness of individual pollinator species. Further research could shed light on these floral shape changes.

Some of the characters associated with male-sterility in *Lycium* (relatively longer calyces and reduced stamens) are reflected in PC3. Values of PC3 for females in the dimorphic species are significantly larger than those of hermaphrodites and all of the cosexual species (Fig. 3C). This reflects the fact that flowers on females of all three dimorphic species have evolved short (i.e., abortive) anthers and long calyces for flowers their size (Fig. 3C; Table 4). In contrast, flowers on hermaphrodites of the dimorphic species are similar to the cosexual species in this component, reflecting stasis in the hermaphrodites of the dimorphic species (Fig. 3C). Size-adjusted calyx tube lengths for dimorphic females are significantly larger than all of the cosexual *Lycium* studied here (Table 4), and reconstruction of calyx tube length indicates

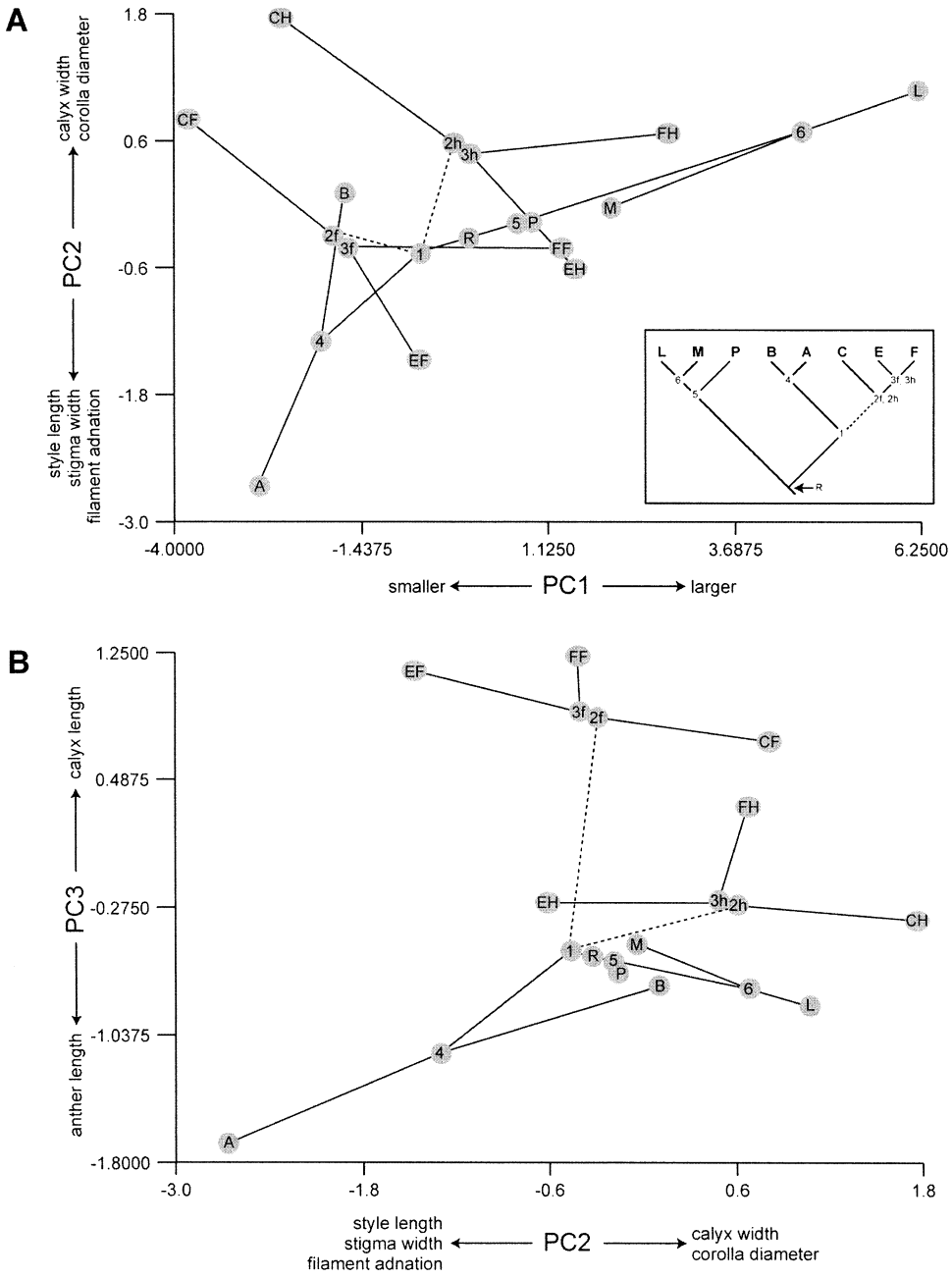

FIG. 5. Plot of the (A) first two principal components and the (B) second and third principal components for females and hermaphrodites in the dimorphic species and cosexual species. Phylogenetic relationships among species (see inset and Fig. 2) are overlaid onto the principal component space. The root of the phylogenetic tree is indicated by the letter R and ancestral nodes (see inset) are indicated by numbers. Ancestral states were inferred using COMPARE (Martins 2001) and incorporated branch-length estimates (see Materials and Methods). The transition to ancestral females and hermaphrodites from their most recent common ancestor with the cosexual species is indicated with dashed lines. Species-morph combinations are: CF, *L. californicum* females; CH, *L. californicum* hermaphrodites; EF, *L. exsertum* females; EH, *L. exsertum* hermaphrodites; FF, *L. fremontii* females; FH, *L. fremontii* hermaphrodites; A, *L. andersonii*; B, *L. berlandieri*; P, *L. parishii*; M, *L. macrodon*; L, *L. pallidum*.

that calyx length increased on flowers of dimorphic females from the common cosexual ancestor (data not shown). Similarly, size-adjusted anther lengths for flowers on dimorphic females are significantly shorter than those on cosexual species and conspecific hermaphrodites (Table 4). Thus, with regard to this cluster of traits, females are the derived gender, and Figure 5 suggests that this shift occurred with, or early after, the origin of gender dimorphism. The functional significance, if any, of enlarged calyces is unknown for *Lycium*, though several hypotheses are reasonable. Flowers of several *Lycium* species, including the dimorphic taxa, are susceptible to Lepidopteran larvae that develop and feed inside developing ovaries and fruits (J. S. Miller, unpubl. obs.). An enlarged calyx could perhaps result in more effective protection

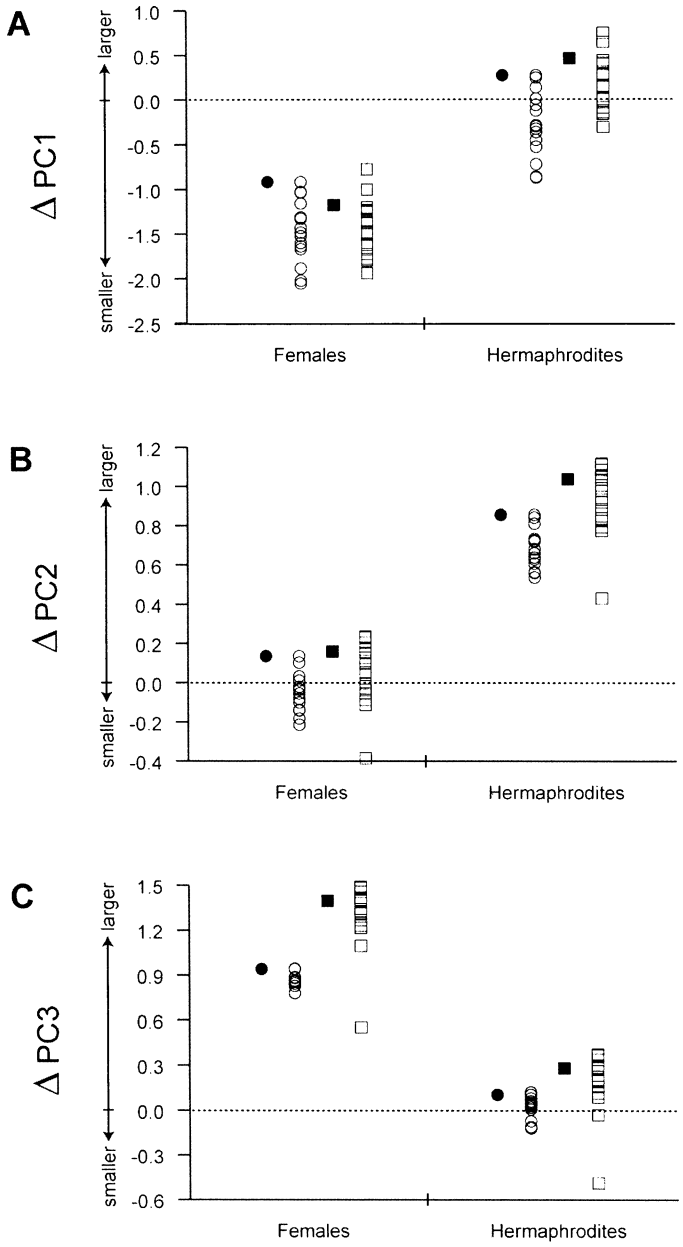

FIG. 6. Sensitivity analysis of size (PC1) and shape (PC2, PC3) changes associated with the evolution of gender dimorphism. Plotted is the difference in the reconstructed ancestral character states for (A) PC1, (B) PC2, and (C) PC3 between the ancestral cosexual taxon (node 1 in Fig. 5) and the initial female (node 2f in Fig. 5) and hermaphrodite (node 2h in Fig. 5). Open symbols are the fifteen possible alternative topologies given by permuting the branching structure for the phylogeny in Figure 2 assuming equal branch lengths (○) and incorporating branch length information (□). Closed symbols represent the most parsimonious topology assuming equal branch lengths (●) or incorporating branch lengths (■). The dotted line represents no change in the ancestral female or ancestral hermaphrodite from the ancestral cosexual taxon.

of ovules and seeds in ovaries and fruits. Alternatively, the longer calyces of flowers on females may function to structurally support developing fruit. It may also be possible that the longer calyces on flowers from female plants are photosynthetic and function to offset the cost of fruit production. For example, in dioecious *Silene latifolia* (Caryophyllaceae), females have larger sepals than males and they retain them for longer periods of time (Carroll and Delph 1996). Because sepals of females fix carbon in this species (Laporte and Delph 1996), it is possible that the larger sepals of females result in greater photosynthesis that enhances fruit and seed production (Carroll and Delph 1996; see also Galen et al. 1993). Experimental exploration of these possibilities for *Lycium* would be an interesting extension of this study.

The Degree of Sexual Dimorphism

Phylogenetic analysis has shown that gender dimorphism has evolved just once in a common ancestor to the three dimorphic species (Miller 2002). Also, Miller and Venable (2002) have shown a similarly low level of female function in the hermaphrodites of the three dimorphic species, suggesting that they are at a similar position along the pathway to dioecy. Thus, it is not surprising that the floral dimorphism was broadly similar among the three dimorphic species, as can be seen graphically in Figs. 3 and 5. Yet, interestingly, there were some significant differences in the degree of floral sexual dimorphism. Overall floral size dimorphism was greatest in *L. exsertum*, being expressed mostly in calyx and corolla width and anther and filament lengths (Fig. 4A). Relative to flower size, *L. exsertum* also has the most dimorphic calyx tube lengths and anther sizes (Fig. 4B). Indeed, *L. exsertum*'s name derives from its large, long-exserted anthers in hermaphrodites (Fig. 2). In contrast, *L. fremontii* had the greatest absolute (Fig. 4A) and floral size independent (Fig. 4B) sexual dimorphism in stigma and style traits. *Lycium fremontii* also has the greatest separation between anther and stigma levels in hermaphrodites. Previous studies have shown that although there is no male function in females of dimorphic species, hermaphrodites, which are self-compatible, often set a few fruits and seeds (Miller 2000; Miller and Venable 2002). Thus, herkogamy (the spatial separation of male and female function within flowers) may have functional relevance for the degree of selfing and interference between gender functions in hermaphrodites, and *L. fremontii* has the greatest degree of herkogamy.

This is the first study to examine changes in floral morphology at and around the evolutionary transition to gender dimorphism using an explicitly phylogenetic context. We argue that this kind of analysis needs to be done more frequently if we are to evaluate the contrasting hypotheses that have been proposed to explain flower size dimorphism in gynodioecious and dioecious species.

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Literature Cited

Ashman, T- L. 1994. Reproductive allocation in hermaphrodite and female plants of *Sidalcea oregana* ssp. *spicata* (Malvaceae) using four currencies. Am. J. Bot. 81:433–438.

———. 1999. Quantitative genetics of floral traits in a gynodioecious strawberry *Fragaria virginiana*: implications for the independent evolution of female and hermaphrodite floral phenotypes. Heredity 83:733–741.

———. 2000. Pollinator selectivity and its implications for the evolution of dioecy and sexual dimorphism. Ecology 81: 2577–2591.

Ashman, T- L., and M. L. Stanton. 1991. Seasonal variation in pollination dynamics of sexually dimorphic *Sidalcea oregana* ssp. *spicata* (Malvaceae). Ecology 72:993–1003.

Baker, H. G. 1948. Corolla size in gynodioecious and gynomonecious species of flowering plants. Proc. Leeds Philos. Soc. 5: 136–139.

Bateman, A. J. 1948. Intrasexual selection in *Drosophila*. Heredity 2:349–369.

Bell, G. 1985. On the function of flowers. Proc. R. Soc. Lond. B 224:223–265.

Bernardello, L. M. 1986. Revisión taxonómica de las especies sudamericanas de *Lycium* (Solanaceae). Bol. Acad. Nac. Cienc. Cordoba 57:173–356.

Carroll, S. B., and L. F. Delph. 1996. The effects of gender and plant architecture on allocation to flowers in dioecious *Silene latifolia* (Caryophyllaceae). Int. J. Plant Sci. 157:493–500.

Charlesworth, D. 1993. Why are unisexual flowers associated with wind pollination and unspecialized pollinators? Am. Nat. 141: 481–489.

Chiang-Cabrera, F. 1981. A taxonomic study of the North American species of *Lycium* (Solanaceae). Ph.D. diss., University of Texas, Austin, TX.

Darwin, C. 1877. The different forms of flowers on plants of the same species. J. Murray, London.

Delph, L. F. 1990. Sex differential resource allocation patterns in the subdioecious shrub *Hebe subalpina*. Ecology 71:1342–1351.

———. 1996. Flower size dimorphism in plants with unisexual flowers. Pp. 217–237 *in* D. G. Lloyd and S. C. H. Barrett, eds. Floral biology. Chapman and Hall, New York.

Delph, L. F., L. F. Galloway, and M. L. Stanton. 1996. Sexual dimorphism in flower size. Am. Nat. 148:299–320.

Donoghue, M. J., and D. D. Ackerly. 1996. Phylogenetic uncertainties and sensitivity analyses in comparative biology. Proc. R. Soc. Lond. B 351:1241–1249.

Eckhart, V. M. 1992. The genetics of gender and the effects of gender on floral characters in gynodioecious *Phacelia linearis* (Hydrophyllaceae). Am. J. Bot. 79:792–800.

———. 1999. Sexual dimorphism in flowers and inflorescences. Pp. 123–148 *in* M. A. Geber, T. E. Dawson, and L. F. Delph, eds. Gender and sexual dimorphism in flowering plants. Springer-Verlag, Berlin.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783–791.

Fukuda, T., J. Yokoyama, and H. Ohashi. 2001. Phylogeny and biogeography of the genus *Lycium* (Solanaceae): Inferences from chloroplast DNA sequences. Mol. Phylogenet. Evol. 19: 246–258.

Galen, C., and M. E. A. Newport. 1987. Bumblebee behavior and selection on flower size in the sky pilot, *Polemonium viscosum*. Oecologia 74:20–23.

Galen, C., and M. L. Stanton. 1989. Bumblebee pollination and floral morphology—factors influencing pollen dispersal in the alpine sky pilot, *Polemonium viscosum* (Polemoniaceae). Am. J. Bot. 76:419–426.

Galen, C., T. E. Dawson, and M. L. Stanton. 1993. Carpels as leaves: meeting the carbon cost of reproduction in an alpine buttercup. Oecologia 95:187–193.

Geber, M. A., T. E. Dawson, and L. F. Delph, eds. 1999. Gender and sexual dimorphism in flowering plants. Springer, Berlin.

Gibson, J. P., and P. K. Diggle. 1997. Structural analysis of female and hermaphroditic flowers of a gynodioecious tree, *Ocotea tenera* (Lauraceae). Am. J. Bot. 84:298–307.

Gilmartin, A. J. 1983. A male-sterile morph in *Lycium fremontii* (Solanaceae) from Baja California. Madrono 30:127–128.

Hasegawa, M., H. Kishino, and T. Yano. 1985. Dating the human-ape split by a molecular clock of mitochondrial DNA. J. Mol. Evol. 22:160–174.

Hitchcock, C. L. 1932. A monographic study of the genus *Lycium* of the western hemisphere. Ann. Mo. Bot. Gard. 19:179–374.

Humeau, L., T. Pailler, and J. D. Thompson. 1999. Cryptic dioecy and leaky dioecy in endemic species of *Dombeya* (Sterculiaceae) on La Reunion. Am. J. Bot. 86:1437–1447.

Johnson, S. G., L. F. Delph, and C. L. Elderkin. 1995. The effect of petal-size manipulation on pollen removal, seed set, and insect-visitor behavior in *Campanula americana*. Oecologia 102: 174–179.

Joubert, A. M. 1981. 'n Taxsonomies-morfologiese studie van *Lycium* in suider-Afrika. M.Sc. thesis, University of the Orange Free State, Bloemfontein, South Africa.

Kearns, C. A., and D. W. Inouye. 1993. Techniques for pollination biologists. University Press of Colorado, Niwot, CO.

Laporte, M. M., and L. F. Delph. 1996. Sex-specific physiology and source-sink relations in the dioecious plant *Silene latifolia*. Oecologia 106:63–72.

Lloyd, D. G. 1982. Selection of combined versus separate sexes in seed plants. Am. Nat. 120:571–585.

Lloyd, D. G., and C. J. Webb. 1977. Secondary sex characters in plants. Bot. Rev. 43:177–216.

Martins, E. P. 2001. COMPARE. Computer programs for the statistical analysis of comparative data. Ver. 4.4. Available from the author at http://compare.bio.indiana.edu/. Dept. of Biology, Indiana University, Bloomington IN.

Miller, J. S. 2000. Selective forces in the evolution of gender dimorphism in *Lycium* (Solanaceae). Ph.D. diss., University of Arizona, Tucson, AZ.

———. 2002. Phylogenetic relationships and the evolution of gender dimorphism in *Lycium* (Solanaceae). Syst. Bot. 27:416–428.

Miller, J. S., and D. L. Venable. 2000. Polyploidy and the evolution of gender dimorphism in plants. Science 289:2335–2338.

———. 2002. The transition to gender dimorphism on an evolutionary background of self-incompatibility: an example from *Lycium* (Solanaceae). Am. J. Bot. 89: *in press*.

Minne, L., J. J. Spies, H. J. T. Venter, and A. M. Venter. 1994. Breeding systems in some representatives of the genus *Lycium* (Solanaceae). Bothalia 24:107–110.

Plack, A. 1957. Sexual dimorphism in Labiatae. Nature 180: 1218–1219.

———. 1958. Effect of gibberellic acid on corolla size. Nature 182: 610.

Puterbaugh, M. N., A. Wied, and C. Galen. 1997. The functional ecology of gynodioecy in *Eritrichum aretioides* (Boraginaceae). Am. J. Bot. 84:393–400.

Renner, S. S., and R. E. Ricklefs. 1995. Dioecy and its correlates in the flowering plants. Am. J. Bot. 82:596–606.

SAS Institute. 1989. SAS/STAT User's Guide. Ver. 6. 4th ed. Vol. 2. SAS Institute, Cary, NC.

Sawyer, N. W., and G. J. Anderson. 2000. Dioecy in South American *Deprea* (Solanaceae). Biotropica 32:291–298.

Swofford, D. L. 2002. PAUP* Phylogenetic analysis using parsimony (*and other methods). Ver. 4.0b10. Sinauer Associates, Sunderland, MA.

Thomson, J. D., and J. Brunet. 1990. Hypotheses for the evolution of dioecy in seed plants. Trends Ecol. Evol. 5:11–16.

Vaughton, G., and M. Ramsey. 1998. Floral display, pollinator visitation, and reproductive success in the dioecious perennial herb *Wurmbea dioica* (Liliaceae). Oecologia 115:93–101.

Venter, A. M., H. J. T. Venter, and R. L. Verhoeven. 1999. Sexuality

in the African *Lycium* (Solanaceae) concluded. Poster presented at the XVI International Botanical Congress, St. Louis, MO.
Weiss, D., and A. H. Halevy. 1989. Stamens and gibberellin in the regulation of corolla pigmentation and growth in *Petunia hybrida*. Planta 179:89–96.
Widén, M., and B. Widén. 1999. Sex expression in the clonal gynodioecious herb *Glechoma hederacea* (Lamiaceae). Can. J. Bot. 77:1689–1698.
Williams, C. F., M. A. Kuchenreuther, and A. Drew. 2000. Floral dimorphism, pollination, and self-fertilization in gynodioecious *Geranium richardsonii* (Geraniaceae). Am. J. Bot. 87:661–669.
Willson, M. F., and J. Ågren. 1989. Differential floral rewards and pollination by deceit in unisexual flowers. Oikos 55:23–29.
Yampolsky, C., and H. Yampolsky. 1922. Distribution of sex forms in the phanerogamic flora. Bibl. Genet. 3:1–62.
Young, H. J., and M. L. Stanton. 1990. Influences of floral variation on pollen removal and seed production in wild radish. Ecology 71:536–547.

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