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For the Special Issue: The Evolutionary Importance of Polyploidy

Polyploidy and sexual system in angiosperms: Is there an association?[1]

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PREMISE OF THE STUDY: Flowering plants display a variety of sexual systems, ranging from complete cosexuality (hermaphroditism) to separate-sexed individuals (dioecy). While dioecy is relatively rare, it has evolved many times and is present in many plant families. Transitions in sexual systems are hypothesized to be affected by large genomic events such as whole-genome duplication, or polyploidy, and several models have been proposed to explain the observed patterns of association.

METHODS: In this study, we assessed the association between ploidy and sexual system (separate or combined sexes). To this end, we assembled a database of ploidy levels and sexual systems for ~1000 species, spanning 18 genera and 15 families. We applied several phylogenetic comparative approaches, including Pagel's coevolutionary framework and sister clade analyses, for detecting correlations between ploidy level and sexual system.

KEY RESULTS: Our results indicate a broad association between polyploidy and sexual system dimorphism, with low evolutionary stability of the diploid-dioecious condition observed in several clades. A detailed examination of the clades exhibiting this correlation reveals that it is underlain by various patterns of transition rate asymmetry.

CONCLUSIONS: We conclude that the long-hypothesized connection between ploidy and sexual system holds in some clades, although it may well be affected by factors that differ from clade to clade. Our results further demonstrate that to better understand the evolutionary processes involved, more sophisticated methods and extensive and detailed data sets are required for both broad and focused inquiry.

KEY WORDS correlated evolution; dioecy; hermaphroditism; polyploidy; sexual dimorphism; sexual system; whole-genome duplication

Sexual diversity is especially evident in angiosperms, with a wide spectrum of sexual systems displayed in many taxa (Yampolsky, 1922; Barrett, 2002; Bachtrog et al., 2014). At one end of this spectrum, individuals are entirely hermaphroditic, bearing only flowers with both functional male and female reproductive organs (i.e., perfect flowers). At the other extreme, sexes are completely separate, with individuals bearing either male or female flowers (dioecy). In between, virtually every combination of perfect and single-sex flowers on individuals and in populations is found, defining sexual systems known as monoecy, gyno- and andromonoecy, gyno- and androdioecy, and others (Table 1; Charnov et al., 1976; Lloyd, 1976; Charlesworth and Charlesworth, 1978; Sakai and Weller, 1999). These sexual systems can also be categorized as sexually monomorphic ones, in which all individuals bear both male and female reproductive structures (e.g., hermaphroditic and monoecious species), and sexually dimorphic ones, in which at least some individuals function only as males or only as females (e.g., dioecy, gynodioecy).

Dioecy is widespread and has originated independently many times during flowering plant evolution. Yet, dioecious species comprise only approximately 6% of flowering plant species (Renner, 2014). Though rare, dioecy is believed to be advantageous under certain evolutionary scenarios. For example, it provides a mechanism to prevent self-fertilization, which can be important to avoid inbreeding depression especially when other such mechanisms are absent (Thomson and Barrett, 1981; Thomson and Brunet, 1990; Freeman et al., 1997; but see Givnish, 1982). Dioecy may also yield more efficient resource allocation, allowing gender-specific strategies and alleviating trade-offs between male and female functions (Charnov et al., 1976; Lloyd, 1976; Charlesworth and Charlesworth, 1978; Montesinos et al., 2006; Tognetti, 2012). Dioecy

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TABLE 1. Common plant sexual systems and their categorization into sexually monomorphic and sexually dimorphic ones.

Sexual system	Description	Sexually dimorphic
Hermaphrodite	All individuals bear flowers that include both male and female structures (perfect flowers).	No
Monoecy	All individuals bear separate male and female flowers.	No
Dioecy	Individuals bear either male or female flowers.	Yes
Gynodioecy	The population consists of both hermaphroditic and female individuals.	Yes
Androdioecy	The population consists of both hermaphroditic and male individuals	Yes
Gynomonoecy	All individuals bear both female and perfect flowers.	No
Andromonoecy	All individuals bear both male and perfect flowers.	No
Polygamodioecy/Trioecy	The population consists of males, females and hermaphrodites.	Yes
Polygamomonoecy	All individuals bear male, female and perfect flowers.	No

may additionally be favored in some ecological settings due to a suite of correlated traits, including abiotic pollination, biotic dispersal, woody growth form, and fleshy fruit (Vamosi et al., 2003; Schwander and Crespi, 2009), although whether these traits are a cause or consequence of sexual system is difficult to determine.

Transitions from hermaphroditism to dioecy may thus be selected for in certain conditions and may occur through one of several possible pathways. Dioecy may evolve through an intermediate phase in which the population is composed of hermaphroditic and female (or male) individuals. This condition, termed gynodioecy (or androdioecy), is followed by the replacement of hermaphrodites with the opposite single-sexed type (Spigler and Ashman, 2012). Therefore, this pathway requires two sterility mutations: a male-sterility mutation creating female individuals, and a female-sterility mutation that results in male individuals (Charlesworth and Charlesworth, 1978). An alternative pathway for the transition from hermaphroditism to dioecy is via monoecy, a sexual system where individuals bear both male-functioning and female-functioning flowers (but not perfect flowers). In this pathway, the final transition to dioecy is mediated by selection toward more extreme sex allocation (Lloyd, 1980). Dioecy may also evolve directly from heterostylous (hermaphrodite) ancestors via reciprocal reductions in male and female function of the style morphs (Lloyd, 1979).

While the mechanisms of transition to dioecy have been extensively studied in various taxonomic groups, transitions away from dioecy have generally received less attention, despite recent research suggesting that such transformations are not infrequent (Alonso and Herrera, 2011; Barrett, 2013; Crossman and Charlesworth, 2014). Nevertheless, it was shown that reversions to hermaphroditism can occur following a population bottleneck in a gynodioecious or androdioecious species, depending on the genetics of sex determination (VanBuren et al., 2015). Transitions to hermaphroditism may also be driven by a combination of sex inconstancy and long-distance dispersal (Baker, 1955; Pannell et al., 2015), allowing establishment in a new location (Case et al., 2008).

Transition rates to and from dioecy may also be altered following large-scale genomic events, such as whole-genome duplication (WGD, or polyploidy). Like sexual system, changes in ploidy are taxonomically widespread and hypothesized to have both genetic and ecological consequences. Polyploidy has occurred multiple times in the evolution of nearly all vascular plant lineages (Jiao et al., 2011), with approximately 35% of extant flowering plant species thought to be of recent polyploid origin (Wood et al., 2009). Polyploids often differ markedly from their diploid progenitors in morphological, physiological, and life history characteristics (Levin, 1983; Ramsey and Schemske, 2002), and these differences may contribute to the establishment and success of polyploid species in novel ecological settings. In particular, the major changes that follow polyploidy may drive shifts in sexual system (reviewed by Ashman et al., 2013).

The association between ploidy and the sexual system was initially mentioned by Baker (1984) and Jennings (1995), and a number of hypotheses have been raised to explain such an association (reviewed by Ashman et al., 2013). These hypotheses can be roughly classified into two types: those consistent with an association of polyploidy and sexual dimorphism or dioecy, and those consistent with an association between polyploidy and hermaphroditism.

Detailed studies of specific groups have provided evidence for transitions from sexually monomorphic diploids to sexually dimorphic polyploids in *Lycium* (Miller and Venable, 2000; Yeung et al., 2005; Blank et al., 2014; Levin et al., 2015; Miller et al., 2015), *Fragaria* (Spigler and Ashman, 2011), and *Leptinella* (Himmelreich et al., 2012). The two transitions involved, one in ploidy and one in sexual system, may occur nearly simultaneously as WGD generally lead to various mutations and changes in gene expression patterns that may cause male or female sterility (Spigler and Ashman, 2011; Zhang et al., 2011). Such sterility conditions provide the raw material for the evolution of sexual dimorphism. An alternative model suggests that transitions in sexual system are mediated by shifts from self-incompatibile to self-compatible mating systems (Stebbins, 1957; Miller and Venable, 2000). This model applies when WGD in a self-incompatible hermaphroditic species causes the loss of self-incompatibility, thereby producing a polyploid species that is self-compatible and hermaphroditic (Barringer, 2007; Husband et al., 2008; Robertson et al., 2011). This self-compatibility state allows for increased selfing that might lead to inbreeding depression. The subsequent selective force in favor of outcrossing may ultimately result in dioecy. Polyploidy may also promote the evolution of dioecy through ecological effects. It is believed that polyploidy facilitates the invasion of new, and sometimes harsh, environments (Hijmans et al., 2007; Ainouche et al., 2009), in which separate sexes may be advantageous for efficient use of resources for reproduction (Armbruster and Reed, 2005; Case and Ashman, 2007). In some cases, dioecious species can invade extreme environments, which their hermaphroditic progenitors cannot colonize (Ashman, 2006). Production of unreduced gametes is highly sensitive to environmental fluctuations (McHale, 1983; De Storme and Geelen, 2014) may therefore increase under extreme conditions encountered in the newly colonized environments.

On the other hand, there is also empirical support for transitions from sexually dimorphic diploids to sexually monomorphic polyploids, specifically in *Mercurialis* (Pannell et al., 2004; Obbard et al., 2006), *Empetrum* (Miller and Venable, 2000), and *Bryonia* (Volz and Renner, 2008). Explanatory models generally rely on the assumption that monomorphic individuals are self-fertile. One such model suggests that the cosexual state promotes the creation of polyploid individuals: if certain individuals produce unreduced gametes more frequently than others, then high self-fertilization rates would increase the chances that two unreduced gametes

would unite and, thus, the probability of autopolyploid formation (Ramsey and Schemske, 1998). A second model focuses on the establishment of polyploid populations. Selfing alleviates the fitness reduction in new polyploids caused by minority cytotype exclusion (Levin, 1975), since selfers can reproduce without the need for a partner, and thus avoid the reproductive barriers between polyploids and their diploid progenitors (Husband and Sabara, 2003). Finally, the ability of polyploids to colonize new ecological regions may create an advantage for selfing hermaphrodites due to selection for reproductive assurance under low population densities (Pannell et al., 2004).

Despite the large number of hypotheses raised to explain the association between ploidy level and the sexual system—two traits with major effects on plant evolution—comparative examination across multiple clades encompassing a wide taxonomic breadth has been surprisingly scarce. It is thus not yet known what pattern of correlation emerges when multiple clades are examined. To this end, we assembled a data set of sexual systems and ploidy levels, which allowed us to examine the association pattern between these two traits across approximately 1000 angiosperm species, spanning 18 genera and 15 families.

MATERIALS AND METHODS

Trait data—A broad data set of sexual systems among angiosperms was obtained from the Tree of Sex Consortium (2014). The data set consists of sexual system information of 5544 angiosperm species in 57 genera, collected from a thorough literature search.

Sexual systems were originally categorized into four states: hermaphroditism, dioecy variants (including gynodioecy, androdioecy, and polygamodioecy), monoecy and its variants (including monoecy, gynomonoecy, andromonoecy, and polygamomonoecy), and dioecy. To reduce the parameter space and to improve the clarity of our analyses, these states were combined to form two sexual system categories, using two criteria: (1) dioecy vs. nondioecy: dioecy was separated from all other states, i.e., classifying hermaphrodites, dioecy variants, monoecy, and its variants to one group, and leaving only purely dioecious species in the other group; (2) sexual system monomorphism vs. sexual system dimorphism (hereafter referred to as sexual monomorphism and sexual dimorphism, respectively, and should not be taken to represent morph differences between male and female flowers)—hermaphrodites, monoecy and its variants were classified into one group (sexually monomorphic), and dioecy and its variants to the other group (sexually dimorphic). Classifying sexual systems in these two manners allows testing different hypotheses regarding their correlation with polyploidy. The first classification is associated with models of correlation that require complete separation of sexes, while the second classification is concerned with the presence of two morphs in the population, where hermaphrodites are still present. The different classifications may also be viewed as two levels of strictness, in which we first define dioecy in its strict meaning, and then in a broader sense that allows for states that are possible precursors to full dioecy to be recognized as steps away from hermaphroditism. Furthermore, if WGD causes immediately only sterility of one sexual function, we would expect a stronger correlation between polyploidy and dimorphism than between polyploidy and strict dioecy.

Species for which the sexual system was not reported were treated as missing data. Additionally, species for which multiple sexual system states were reported (e.g., "hermaphrodite *or* monoecious") were classified to one of the two states only if the polymorphism could be resolved without conflicts, in the context of each classification method. Otherwise, such species were treated as missing data. For example, a species reported as "monoecious or gynodioecious" would be classified as nondioecious under the dioecy classification method, but would be classified as missing data under the sexual dimorphism classification method.

To obtain ploidy level inferences, we extracted chromosome numbers from the Chromosome Counts Database (CCDB; Rice et al., 2015). When multiple counts were reported for a given taxon, the median number was used. The median was used since it is more robust to errors that may occur due to taxonomic ambiguities, errors in the process of chromosome-number determination, and in the parsing procedures of CCDB. Chromosome number evolution was analyzed in each genus separately using 100 phylogenies randomly sampled from the posterior tree distribution (see details below regarding the phylogenetic reconstruction). For each genus, species-specific ploidy level inferences were based on a variant of the ploidy inference pipeline of the chromEvol program described in Glick and Mayrose (2014). This likelihood-based method estimates the expected number of polyploidy and dysploidy transitions along each branch of the phylogeny, thereby allowing explicit categorization of terminal taxa to either diploid or polyploid relative to other taxa in the group examined. According to this classification, polyploids are explicitly defined as those taxa that had undergone a polyploidization event sometime since divergence from the most recent common ancestor (MRCA) of the group examined (here, genus-level phylogenies). Accordingly, a lineage that has undergone a polyploidy event after divergence from the MRCA but has since diploidized is still classified as a polyploid. We also note that the chromEvol model (and the reconstructed phylogeny) does not allow us to distinguish between WGDs associated with hybridization (allopolyploidy) and those that are not (autopolyploidy). The method assesses the inference reliability by applying a simulation-based approach and assigning an appropriate reliability score to each taxon (Appendix S1 in Supplemental Data with the online version of this article). A taxon whose ploidy-level inference reliability score was below 0.95 was deemed unreliable and treated as missing data. We excluded 333 taxa (all but four without an assigned chromosome number) due to unreliable ploidy inference (see online Appendices S2–S4). A detailed description of the ploidy inference procedure and the results of the procedure are provided in Appendices S1 and S4, respectively. Due to the complexity of the chromosome number evolution of the genus *Rumex*, a customized analysis scheme was applied to acquire reliable ploidy inferences in this genus (Appendix S1).

Database assembly—A name resolution procedure was applied to all species names to account for synonymous taxon names, misspellings, and differences in naming conventions using the Taxonome software (Kluyver and Osborne, 2013). The underlying reference database for names was a local repository of synonymous and accepted names that was created based on The Plant List (V1.1; http://www.theplantlist.org/) and Solanaceae Source (http://solanaceaesource.org/). Genera for which chromosome numbers were available for fewer than 10 species or for which trait data were available for fewer than 15 species were removed. Genera for which low ploidy inference reliability measures (see Appendix S1) were obtained were discarded as well. The assembled database contained

991 angiosperm species when sexual systems were categorized by the sexual dimorphism criterion and 911 species using the dioecy criterion. These species belong to 18 and 16 genera, respectively (Table 2; Appendix S3).

Phylogenetic reconstruction—To allow for an analysis within an evolutionary framework, we applied a computational pipeline to reconstruct the phylogeny of each genus analyzed as detailed in online Appendix S5. Briefly, the pipeline followed these general steps: First, for each genus, all DNA sequences available in GenBank (Benson et al., 2013) were retrieved. Sequences were clustered into orthology groups (roughly representing genomic loci) using the orthoMCL software (Li et al., 2003), and an appropriate outgroup to root the phylogeny was selected. Next, sequences in each orthology group (cluster) were aligned using the MAFFT program (Katoh and Standley, 2013), and GUIDANCE (Penn et al., 2010) was applied to the resulting multiple sequence alignment (MSA) of each cluster to discard sequences and positions that reduce the MSA reliability. The best-supported model of sequence evolution was determined for each locus and each genus separately, using the program MrAIC (Nylander, 2004). The MSAs for multiple clusters were concatenated, and the result was used as input for a partitioned Bayesian phylogenetic reconstruction using the program MrBayes v3.2.1 (Ronquist and Huelsenbeck, 2003; Ronquist et al., 2012). A relaxed-clock model was used to ensure that the resulting trees were ultrametric. One hundred phylogenies were randomly sampled from the posterior distribution and used in subsequent analyses. The MSAs used for phylogenetic reconstruction and the resulting phylogenies are provided in online Appendices S6 and S7, respectively.

Sister clade analysis—The method described by Read and Nee (1995) was implemented using the R programming language and applied to each of the genera. This nonparametric approach allows the detection of correlated evolution between traits with discrete states by identifying informative independent contrasts. Sister clades differing in their states for both the investigated traits were recorded and then statistically analyzed.

Given a phylogenetic tree, each tip was assigned to one of four possible ordered pairs: (0,0), (0,1), (1,0) or (1,1), where (0,0) represents a diploid, nondioecious (or sexually monomorphic) species, (1,1) represents a polyploid, dioecious (or sexually dimorphic) species, and so on. To perform the analysis, one must specify one of the traits (either the ploidy trait in position 1 or the sexual system trait in position 2) to be the independent factor, while the other trait is considered dependent. For maximizing the number of identified contrasts, and since no specific expectation regarding the causality of correlation was assumed, the search was performed twice, each time assuming a different trait to be the independent one. The two sets of contrasts were then merged, while ensuring that each extant taxon appeared in one contrast only. To obtain informative sister clades, a post-order tree search, beginning at the tips of the tree, was conducted. Sister clades of increasing size were examined, in search of sister taxa contrasting in their states of the independent trait. Once an informative clade was detected, it was recorded and pruned from the tree. This procedure ensures that informative clades extracted from the tree are independent and thus can be treated as distinct evolutionary transitions. Once all clades containing a contrast in the independent trait were extracted, they were each examined again, this time checking for contrasts in the dependent trait. The majority state (either 0 or 1) of the dependent trait for each of the two sister taxa was determined, along with the fraction of tips with this particular state. Clades for which this fraction exceeded 0.75 for both sister taxa were assigned the majority states, and if these were contrasting, the clade was deemed informative.

When considering two binary traits, only two types of contrasts are possible: (0,0) vs. (1,1), termed type I contrasts, or (1,0) vs. (0,1), termed type II contrasts. Each clade found to be informative was thus categorized to either type of contrast, and the total number of contrasts of each type was recorded. We accounted for phylogenetic uncertainty by performing the analysis on 100 phylogenies and taking the median number of contrasts of each type. The total number of contrasts of each type over all data sets was compared with the number expected by chance by applying a two-tailed exact binomial test, with contrasts assumed to be of type I or type II with equal probabilities. This procedure was applied both for the sexual dimorphism–sexual monomorphism categorization and the dioecy–nondioecy categorization. In the latter, the *Mammilaria* and *Sidalcea* data sets were excluded due to the lack of sexual system data for these genera.

Independence of evolutionary rates—To examine patterns of coevolution between traits, we applied the method first described by Pagel (1994) as implemented in the BayesTraits program (Pagel and Meade, 2006). Each data set was analyzed twice, corresponding to the two different sexual system categorizations (dioecy–nondioecy and sexual dimorphism–sexual monomorphism); due to lack of data in *Mammilaria* and *Sidalcea*, only the sexual dimorphism–sexual monomorphism analysis could be performed. Detection of correlated

TABLE 2. Genera used in correlation analyses. *N*, estimated number of species in the genus as obtained in the referenced study; NP, number of species in the analyzed phylogeny; NT, number of species in the analyzed phylogeny for which both ploidy inferences and sexual system data are available.

Family	Genus	*N* (Source)	NP	NT
Casuarinaceae	*Allocasuarina*	58 (Steane et al., 2003)	42	27
Asparagaceae	*Asparagus*	200 (Kubota et al., 2012)	30	24
Amaranthaceae	*Atriplex*	300 (Kadereit et al., 2010)	71	34
Rosaceae	*Fragaria*	24 (DiMeglio et al., 2014)	16	14
Oleaceae	*Fraxinus*	43 (Hinsinger et al., 2013)	41	35
Rubiaceae	*Galium*	400 (Mitova et al., 2002)	112	75
Asteraceae	*Leptinella*	42 (Himmelreich et al., 2012)	29	21
Solanaceae	*Lycium*	88 (Levin et al., 2011)	61	57
Cactaceae	*Mammillaria*	145 (Butterworth and Wallace, 2004)	117	84
Poaceae	*Poa*	575 (Gillespie and Soreng, 2005)	107	55
Rosaceae	*Potentilla*	400 (Dobeš and Paule, 2010)	85	15
Rosaceae	*Rubus*	750 (Alice and Campbell, 1999)	101	14
Polygonaceae	*Rumex*	200 (Talavera et al., 2011)	35	26
Malvaceae	*Sidalcea*	25 (Andreasen and Baldwin, 2003)	23	16
Caryophyllaceae	*Silene*	700 (Ghahremaninejad et al., 2014)	186	97
Solanaceae	*Solanum*	1500 (Weese and Bohs, 2007)	431	291
Ranunculaceae	*Thalictrum*	196 (Soza et al., 2012)	91	59
Caprifoliaceae	*Valeriana*	176 (Bell and Michael, 2005)	101	47

evolution was first performed by fitting two models to the data: an independent model, in which polyploidy and the sexual system evolve independently across the phylogeny, and a dependent model, in which a correlation of the two traits is accounted for (Fig. 1). In both models, the root was fixed to diploidy, and transitions from polyploidy to diploidy were not allowed ($q^{N}_{PP\to DP} = q^{D}_{PP\to DP} = 0$; see Fig. 1 for the transition parameter notations) since the process of diploidization occurs over much longer time scales. The model of noncorrelated (independent) evolution is nested within the dependent model when $q^{DP}_{N\to D} = q^{PP}_{N\to D}, q^{DP}_{D\to N} = q^{PP}_{D\to N}, q^{N}_{DP\to PP} = q^{D}_{DP\to PP}$.

In each analysis, parameters of each of the models were optimized within the maximum likelihood (ML) paradigm using 10 different starting point values for each estimated parameter. The two alternative models were optimized independently for a set of 100 Bayesian phylogenies, sampled randomly for each genus. A likelihood ratio (LR) score was calculated per phylogeny as $LR = 2\left[\log L(D) - \log L(I)\right]$, where $L(D)$ and $L(I)$ are the ML estimates of the dependent and independent models, respectively. The median LR score across the 100 phylogenies was used as the representative value (identical results were obtained using the mean instead of the median). We first assumed that the LR follows a χ^2 distribution, with df = 3 (difference in number of parameters between the models), and we used this approximation to test whether the null model of independent evolution can be rejected in favor of the dependent model. To account for multiple comparisons caused by testing for significance in multiple genera, the false discovery rate (FDR; Benjamini and Hochberg, 1995) procedure was applied.

To address statistical concerns about the χ^2 approximation underlying the LR model comparisons, we also applied a parametric bootstrapping approach. We compared the observed LR values against those obtained using simulated trait data on the same set of empirical phylogenies. For each genus, the transition rates and root state probabilities estimated by running the independent model over each of the 100 phylogenies were used to simulate 1000 trait data sets (10 simulations per phylogeny) with the MK2 model, implemented in the asr.stoch function from the Diversitree package in R (FitzJohn, 2012). Similar to the analysis of the true data, only simulated data sets that included some variability in both traits were kept; the procedure was repeated until 1000 data sets were obtained. Simulated data sets were analyzed following the same procedure described above, to obtain a null distribution of the LR statistics. For each genus, the fraction of simulated LRs larger than the LR observed on the real data were calculated and referred to as the *P* value. To ensure further that our simulated data are relevant to the real data we analyzed, we created an additional set of 1000 simulated data sets in which simulated realizations were kept only if the percentage of tips within each state differed by less than 10% from that observed in the original data. We denote these two approaches as unconstrained and constrained tip ratio.

Finally, as an alternative to the ML paradigm, a model choice was also carried out using a Markov chain Monte Carlo (MCMC) approach, which resulted in identical models selected. All results reported in the main text are based on the ML analysis while the details regarding the MCMC analysis are reported in online Appendices S8 and S9.

It was recently revealed that the Pagel (1994) test for correlated evolution does not impose strict phylogenetic independence when assessing statistical significance (Maddison and FitzJohn, 2014). It is therefore prone to reporting a significant correlation even when transitions are so rare that there is no biological support for the idea of correlated evolution. Our parametric bootstrapping approach indirectly addresses this concern by adjusting the significance threshold based on the estimated transition rates. To address this concern more directly, we inferred the number of transitions in each of the traits for each genus by running a stochastic mapping procedure (Huelsenbeck et al., 2003; online Appendix S10) across 100 phylogenies and taking the median number of transitions as a representative. As reported in online Appendix S11, the mean number of transitions per genus was 12.3 and 11.3 under the sexual dimorphism and dioecy categorization, respectively, with a minimum of five transitions per genus (in *Allocasuarina* and *Fraxinus*), indicating that our inferences are not based on very few transitions. However, as discussed by Maddison and FitzJohn (2014), data sets in which the number of transitions in either of the traits is very low may represent the "unreplicated bursts" scenario rather than correlated evolution.

Data sets in which statistically significant evidence for correlated evolution were found were subject to a post hoc analysis, aimed at identifying the factors that contribute the most to the enhanced fit of the dependent model. Specifically, three measures were defined to assess the directionality of inequality between pairs of parameter values, using parameter values inferred under the dependent model:

$$Q_d = \frac{q^{PP}_{N\to D}}{q^{DP}_{N\to D} + q^{PP}_{N\to D}}, Q_h = \frac{q^{PP}_{D\to N}}{q^{DP}_{D\to N} + q^{PP}_{D\to N}}, \text{and}$$

$$Q_p = \frac{q^{D}_{DP\to PP}}{q^{N}_{DP\to PP} + q^{D}_{DP\to PP}}$$

Values of Q_d deviating from 0.5 thus represent a tendency to transition more rapidly from nondioecy (or sexual monomorphism) to dioecy (or sexual dimorphism) under a certain background (diploidy, when Q_d approaches zero, or polyploidy, when it approaches one).

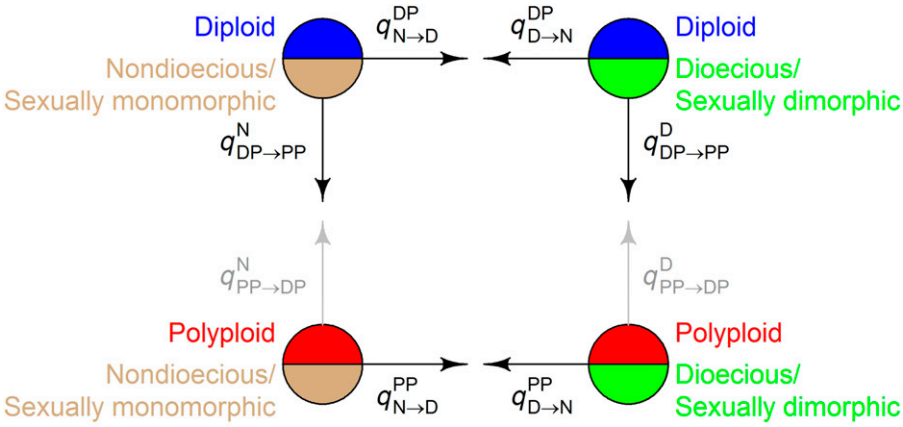


FIGURE 1 A schematic representation of the coevolutionary model for polyploidy and sexual system used in analysis of the independence of evolutionary rates. The rate parameter notations, $q^{B}_{S1\to S2}$, represent a transition from state S1 to state S2 in one trait on the background state, B, of the second trait. N, nondioecy (brown); D, dioecy (green); DP, diploid (blue); PP, polyploid (red).

Similarly, Q_h represents the relative transition rate toward nondioecy (or sexual monomorphism) on a polyploid vs. diploid background, and Q_p represents the relative transition rate toward polyploidy on dioecy relative to nondioecy backgrounds. Each measure was averaged across 100 phylogenies.

Finally, the relative persistence of each of the four character states was calculated. The persistence of a state is defined as the inverse of the sum of all rates away from that state, representing the expected waiting time until a transition occurs. Because raw persistence values cannot be compared across clades, due to the lack of absolute divergence times, we obtained a relative persistence measure between 0 and 1 by dividing each score by the sum of all persistence scores for a given phylogeny. The mean score across 100 phylogenies was taken to represent the persistence of each state. Persistence scores are indicative of the evolutionary stability of combinations of states and are thus useful as an intuitive measure for exploring patterns of trait correlation. A combination with high persistence indicates a tendency for two states to continuously cooccur, which could be taken as evidence that the combination is evolutionarily stable. This measure brings a unique perspective to the analysis. For example, if the polyploidy–dioecy association characterizes a certain clade, it is not obvious whether that is due to the more common occurrence of polyploidy and dioecy or of diploidy and nondioecy. The persistence measure can separate these possibilities.

RESULTS

We assembled sexual system and ploidy-level information for 991 species across 18 genera under the sexual monomorphism–sexual dimorphism classification (online Appendix S12). When the phylogenetic context was ignored and all species of all genera were pooled, approximately 60% were diploid and sexually monomorphic, 14% were diploid and sexually dimorphic, 17% were polyploid and sexually monomorphic, and 9% were polyploid and sexually dimorphic (Table 3A). A statistical test indicated a strong association between the traits, with over-abundance of species in the diploid sexually monomorphic and polyploid sexually dimorphic groups ($P < 10^{-4}$, Fisher's exact test). Similarly, under the nondioecy–dioecy categorization, of 911 species spanning 16 genera (Appendix S12), 62% were diploid and nondioecious, 9% were diploid and dioecious, 23% were polyploid and nondioecious, and 6% were polyploid and dioecious (Table 3B), again yielding a statistical over-abundance of species in the diploid nondioecy and polyploid dioecy groups ($P = 0.01$, Fisher's exact test). An assumption of the above test is that the data are independent, which is obviously violated due to the shared evolutionary history of the analyzed species. We thus used phylogenetic tests to account for species relationships.

Sister clade analysis—Using the sister clade analysis approach, we identified 15 contrasts across the whole data set when sexual systems were categorized as sexually monomorphic vs. sexually dimorphic. Of these, 13 were of type I (sexually monomorphic diploids vs. sexually dimorphic polyploids) and only two were of type II (sexually monomorphic polyploids vs. sexually dimorphic diploids; found only in *Rubus*), leading to a highly significant deviation from the expected 0.5 ratio when applying an exact binomial test ($P = 0.007$). These 15 contrasts came from 10 of the 18 analyzed clades, while in eight genera no contrasts involving transitions in both traits were found (Table 4). A similar trend, albeit statistically nonsignificant ($P = 0.23$), was observed under the dioecy–nondioecy classification with eight of the 11 contrasts identified being of type I (nondioecy diploids and dioecy polyploids) and three of type II (nondioecy polyploids vs. dioecy diploids).

TABLE 3. Numbers of taxa in each trait configuration for the nonphylogenetic association analysis between polyploidy and (A) sexual dimorphism or (B) dioecy. Numbers in parentheses represent the expected number of taxa, with the sign representing overabundance (+) or underabundance (–). SM, sexual monomorphism; SD, sexual dimorphism; N, nondioecy; D, dioecy; DP, diploidy; PP, polyploidy.

A) Numbers of taxa in each trait configuration for the nonphylogenetic association analysis between polyploidy and sexual dimorphism.

Ploidy level / Sexual system	SM	SD
DP	594 +(561)	135 –(168)
PP	169 –(202)	93 +(60)

B) Numbers of taxa in each trait configuration for the nonphylogenetic association analysis between polyploidy and dioecy.

Ploidy level / Sexual system	N	D
DP	568 +(555)	86 –(99)
PP	205 –(218)	52 +(39)

Dependencies in transition rates—Pagel's method was used to investigate whether rates of evolution in ploidy or sexual system depend on the state of the other trait. Under the sexual dimorphism categorization, five of the 18 clades examined had a statistically significant association between sexual dimorphism and polyploidy (*Lycium*, *Leptinella*, *Mammillaria*, *Rubus*, and *Fragaria*; Table 5), which is significantly higher than chance expectation (at $\alpha = 0.05$ we would expect a significant result in roughly one clade; $P = 0.0015$, exact binomial test). Following the FDR correction for multiple testing, the significance of two of the five data sets became

TABLE 4. Median numbers of contrasts across the 100 phylogenies analyzed in different genera, according to the sister clade analysis.

	Dioecy		Dimorphism	
Genus	Type I contrasts	Type II contrasts	Type I contrasts	Type II contrasts
Allocasuarina	0	0	0	0
Asparagus	0	0	0	0
Atriplex	1	0	1	0
Fragaria	3	0	2	0
Fraxinus	1	0	0	0
Galium	0	0	0	0
Leptinella	0	0	0	0
Lycium	2	0	3	0
Mammillaria	—	—	2	0
Poa	0	0	0	0
Potentilla	0	0	0	0
Rubus	0	2	0	2
Rumex	0	0	1	0
Sidalcea	—	—	1	0
Silene	0	0	1	0
Solanum	0	0	0	0
Thalictrum	1	0	1	0
Valeriana	0	1	1	0
Total	**8**	**3**	**13**	**2**

TABLE 5. Summary of results of the analysis of evolutionary rate independence. LR is the median likelihood ratio obtained over 100 phylogenies; *P*, *P* value of the likelihood ratio test between the independent and dependent models, assuming the χ^2_3 approximation; PBS *P*, the *P* value obtained by applying the parametric bootstrapping approach. Significant *P* values following the FDR procedure are marked with a *.

	Dioecy			Dimorphism		
Genus	**LR**	***P***	**PBS *P***	**LR**	***P***	**PBS *P***
Allocasuarina	6.11	0.10	0.13	6.11	0.11	0.16
Asparagus	0.45	0.93	0.99	0.45	0.93	0.99
Atriplex	6.26	0.1	0.17	6.31	0.1	0.17
Fragaria	7.79	0.05	0.02	10.3	0.02	4×10^{-3} *
Fraxinus	7.25	0.06	0.06	0.62	0.89	0.9
Galium	4.87	0.18	0.25	4.29	0.23	0.33
Leptinella	20.70	$<10^{-3}$ *	$<10^{-3}$ *	13.3	4×10^{-3}*	4×10^{-3} *
Lycium	19.10	$<10^{-3}$ *	10^{-3} *	27.6	$<10^{-3}$*	$<10^{-3}$ *
Mammillaria	—	—	—	13.7	3×10^{-3}*	2×10^{-3} *
Poa	3.83	0.28	0.41	5.38	0.15	0.22
Potentilla	5.06	0.17	0.17	5.99	0.11	0.09
Rubus	11.20	0.01	4×10^{-3} *	10.8	0.01	6×10^{-3} *
Rumex	1.39	0.71	0.95	4.05	0.26	0.41
Sidalcea	—	—	—	4.04	0.26	0.29
Silene	1.47	0.69	0.75	1.36	0.71	0.83
Solanum	1.28	0.73	0.73	1.28	0.73	0.76
Thalictrum	5.34	0.15	0.17	5.36	0.15	0.19
Valeriana	3.07	0.38	0.66	4.78	0.19	0.47

only marginal (*Rubus* and *Fragaria*; $P = 0.057$ and $P = 0.058$, respectively).

Similar to the sister-clade comparison, the association is less evident when sexual systems were categorized into nondioecy and dioecy. Three of the 16 clades were significantly associated (*Lycium*, *Leptinella*, and *Rubus*, before FDR correction), which is again higher than the expected number at $\alpha = 0.05$ ($P = 0.043$). Two other clades (*Fragaria* and *Fraxinus*) had a nearly significant association.

The results were highly similar whether significance was assessed by applying the χ^2_3 approximation to the LR distribution or by utilizing the parametric bootstrapping approach (Table 5). However, analysis of the simulations generally indicated that the χ^2 approximation is in many cases not valid (see Appendices S13 and S14). Constraining the tip ratios in the simulated data while applying the parametric bootstrapping approach slightly decreased the *P* values, but overall results did not change, with the same genera showing significant correlation.

Post hoc analyses were carried out on the five genera that had evidence for correlated evolution between ploidy-level and sexual system to investigate the factors influencing this association. Results are summarized below and in Table 6 and Fig. 2.

Lycium—When the correlation between sexual dimorphism and polyploidy was examined, Q_h received a value of 0, indicating a much higher tendency of diploids over polyploids to transition to sexual monomorphism. Similarly Q_p received a value of 1 due to the negligible rate of polyploidization on the sexually monomorphic background ($q^{N}_{DP\rightarrow PP}$). The state where polyploidy and sexual dimorphism co-occur is an absorbing state, with very low rate of transition away from it. All other states were relatively unstable, especially due to rapid transitions away from diploid sexually dimorphic taxa ($q^{D}_{DP\rightarrow PP}$ and $q^{DP}_{D\rightarrow N}$).

When categorizing sexual systems into dioecy and nondioecy, a high value of Q_p indicated higher rates of polyploidization in dioecious lineages than in nondioecious ones, and the most persistent states were those with a polyploid background; the low persistence of the diploid states stemmed from the high transition rates from nondioecy and particularly from dioecy on the diploid background ($q^{D}_{DP\rightarrow PP}$ and $q^{DP}_{D\rightarrow N}$).

TABLE 6. Values of illustrative statistics used in post hoc analyses of five clades in which correlated evolution between polyploidy and sexual system was detected.

Genus	**Sexual system categorization**	Q_d	Q_h	Q_p
Lycium	Dimorphism	0.56	0	1
	Dioecy	$<10^{-3}$	0	0.99
Fragaria	Dimorphism	0.58	$<10^{-3}$	0.9
	Dioecy	$<10^{-3}$	$<10^{-3}$	0.93
Rubus	Dimorphism	0.97	$<10^{-3}$	1
	Dioecy	0.97	$<10^{-3}$	1
Leptinella	Dimorphism	0.92	0.99	0.98
	Dioecy	0.98	1	0.98
Mammillaria	Dimorphism	1	0.57	$<10^{-3}$

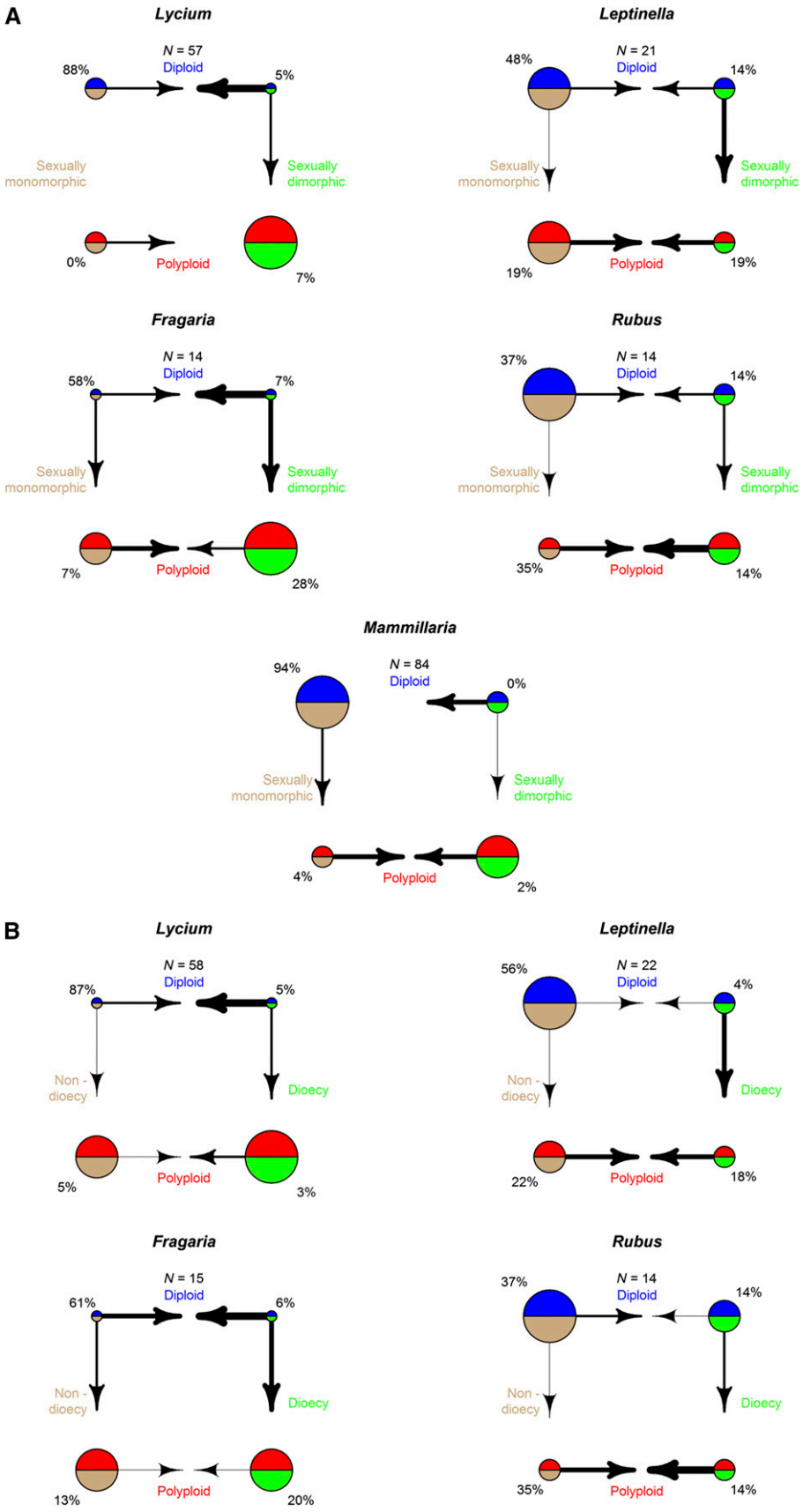
Leptinella—Transitions between sexual systems, in both directions, were found to be faster in polyploids than in diploids (as indicated by high Q_d and Q_h), while the rate of polyploidization was higher on a sexually dimorphic background (high Q_p). The most persistent states were the sexually monomorphic ones, with high transition rates away from sexual dimorphism both by changes in sexual system ($q^{PP}_{D\rightarrow N}$ and $q^{DP}_{D\rightarrow N}$) and polyploidization ($q^{D}_{DP\rightarrow PP}$).

A similar trend was revealed when sexual systems were categorized by the dioecy criterion although under this categorization the diploid, nondioecy state was the single most persistent state. High transition rates to and from dioecy, among polyploid taxa ($q^{PP}_{N\rightarrow D}$ and $q^{PP}_{D\rightarrow N}$) and high polyploidization rates in dioecious taxa ($q^{D}_{DP\rightarrow PP}$) led to low persistence of other trait configurations.

Fragaria—A low Q_h value indicated that transitions toward sexual monomorphism were faster on a diploid background ($q^{DP}_{D\rightarrow N} > q^{PP}_{D\rightarrow N}$). Additionally, high Q_p values indicated higher polyploidization rate on a sexually dimorphic background ($q^{N}_{DP\rightarrow PP} < q^{D}_{DP\rightarrow PP}$). Both diploid states were highly transient, with rapid transitions between sexual systems as well as high polyploidization rates. The polyploid, sexually dimorphic state is the most persistent while the polyploid, sexually monomorphic configuration is also somewhat persistent, although with a high transition rate to sexual dimorphism ($q^{PP}_{N\rightarrow D}$).

Under the dioecy categorization, a similar trend was observed, with Q_h and Q_p displaying similar values to those obtained under the previous categorization. However, the value of Q_d was reduced, indicating $q^{DP}_{N\rightarrow D} > q^{PP}_{N\rightarrow D}$. Again, the two diploid states were highly transient while the two polyploid states were more persistent.

Rubus—Applying either of the sexual systems categorizations, the high value of Q_d and Q_p indicated that transitions to sexual dimorphism (or dioecy) occurred more often on polyploid than on diploid backgrounds and that polyploidization events tended to occur on a sexually dimorphic/dioecious background, respectively. The low value of Q_h is interpreted as a bias toward transitions to sexual monomorphism/nondioecy on polyploid background. The diploid, sexually monomorphic/nondioecious configuration was the most persistent. Other states were much less stable, with high rates of


A
Lycium
N = 57
Diploid
88%
5%
Sexually monomorphic
Sexually dimorphic
0%
Polyploid
7%
Leptinella
N = 21
Diploid
48%
14%
Sexually monomorphic
Sexually dimorphic
19%
Polyploid
19%
Fragaria
N = 14
Diploid
58%
7%
Sexually monomorphic
Sexually dimorphic
7%
Polyploid
28%
Rubus
N = 14
Diploid
37%
14%
Sexually monomorphic
Sexually dimorphic
35%
Polyploid
14%
Mammillaria
N = 84
Diploid
94%
0%
Sexually monomorphic
Sexually dimorphic
4%
Polyploid
2%
B
Lycium
N = 58
Diploid
87%
5%
Non - dioecy
Dioecy
5%
Polyploid
3%
Leptinella
N = 22
Diploid
56%
4%
Non - dioecy
Dioecy
22%
Polyploid
18%
Fragaria
N = 15
Diploid
61%
6%
Non - dioecy
Dioecy
13%
Polyploid
20%
Rubus
N = 14
Diploid
37%
14%
Non - dioecy
Dioecy
35%
Polyploid
14%

transitions away from the diploid, sexually dimorphic/dioecious state ($q^{PP}_{D\to N}$ and $q^{D}_{DP\to PP}$) and transitions in sexual systems among the polyploid states ($q^{PP}_{N\to D}$ and $q^{PP}_{D\to N}$).

Mammillaria—Q_d was high, indicating that $q^{DP}_{N\to D} < q^{PP}_{N\to D}$. Polyploidization rates were higher in sexually monomorphic than in sexually dimorphic states, as demonstrated by the low Q_p value. The polyploid, sexually dimorphic and diploid, sexually monomorphic states were the most persistent states.

DISCUSSION

Here, we examined the longstanding hypothesis of correlated evolution of ploidy and sexual system in flowering plants. Many models have been suggested to explain this possible association. In this study, we did not aim at examining a particular model, but rather applied a more general approach to observe which patterns of association, if any, are supported by the data. We used a broad data set to explore patterns of co-occurrence between these two traits, utilizing both nonphylogenetic and phylogenetic approaches, across multiple angiosperm genera. Despite the particularities of each genus examined and the extensive ecological, geographical, and historical differences among them, the assembled data and the methods we used allowed us to reveal a general association in which polyploidy and sexual dimorphism occurred together more often than expected by chance. This result is robust to various methodological approaches of correlation detection and data manipulation (but see the later discussion regarding caveats of the methods). However, this association does not occur in all clades, and even within groups displaying correlated evolution, a variety of evolutionary scenarios was found.

By merely examining the distribution of sexual systems and ploidy levels in the analyzed data set, disregarding any phylogenetic relationships between the taxa, it is understandable why hypotheses regarding the association between the two traits were originally raised since polyploid dioecious (or sexually dimorphic) species are over-represented in the data set. However, this result might be the consequence of acquisition bias, since we considered only genera that include variation in both ploidy and sexual systems. Moreover, ignoring the shared ancestral origins of the examined clades may lead to inherent biases in assessing the extent to which these two traits have coevolved.

To address this latter concern, we applied two phylogenetic approaches for testing correlated evolution. When Pagel's (1994) modeling approach was used to analyze each clade independently, the fraction of genera displaying evidence for correlated evolution significantly exceeded what would be expected by chance, regardless of the categorization mode of sexual systems. Our follow-up analysis of the five clades in which a signal of coevolution was detected, revealed that no single evolutionary pattern characaterizes all five clades. In *Lycium* and *Fragaria*, the most persistent state is the one in which both sexual dimorphism and polyploidy occur. However, in *Rubus*, the sexually monomorphic, diploid state is more persistent (which is also consistent with the sister clade analysis results), while in *Mammillaria* and *Leptinella* no clear trend was evident, although these two genera had markedly different patterns. The trends of some clades changed when applying the dioecy–nondioecy categorization system. A common characteristic of all clades is a high transition rate away from the sexually dimorphic (or strictly dioecious) diploid state, again supporting the overall trend, although weak, toward the positive association between polyploidy and dioecy (and its variants). It should be noted that results of the persistence analyses may be biased due to the definition of polyploidy used in this study. Species were denoted polyploids if they had undergone a WGD since divergence from the root of the genus phylogeny, regardless of possible diploidization that might have occurred subsequently. Therefore, persistence scores of the polyploid states may be inflated merely because backward transitions were not modeled.

Results of the sister clade analysis indicated a significant association between polyploidy and sexual dimorphism, though not between polyploidy and dioecy per se. This difference cannot be explained solely by the two additional genera which were analyzed under the dimorphism—but not the dioecy—categorization (*Mammillaria* and *Sidalcea*), because statistical significance remained when these two groups were removed from the dimorphism analysis (10 type I contrasts, 2 type II contrasts, $P = 0.02$). In four of the clades examined (*Lycium*, *Rumex*, *Silene*, and *Valeriana*), different numbers (or types) of contrasts were detected under the two categorization methods. For example, the species *Lycium fremontii* and *L. exsertum* are polyploid and gynodioecious, and therefore classified as nondioecious (1,0), but sexually dimorphic (1,1). Together, they form a (1,1) clade that contrasts with its (0,0) sister clade, but only when applying the dimorphism categorization. This example, as well as other cases examined, indicates that the difference in outcome of the test when using different sexual system classifications is biologically meaningful: the association between polyploidy and sexual dimorphism is more evident than the pattern for strict dioecy. One biological explanation for this is the hypothesized effect of polyploidy on the transition to gynodioecy—a sexual system categorized here as sexually dimorphic but nondioecious. The majority of natural gynodioecy is thought to arise from complex sex determination mechanisms in which cytoplasmic male sterility (CMS) genes interact with nuclear genes that restore fertility (Bailey and Delph, 2007). Hybridization can lead to cytonuclear discordance (Bock et al., 2014), so allopolyploids may have cytonuclear interactions controlling sex determination (Freeling, 2009). Such interactions may cause polyploid species to transition to gynodioecy but would not apply to transitions all the way to dioecy.

←

FIGURE 2 The coevolutionary genus-specific patterns inferred for genera that significantly deviated from independent evolution of the two traits. Width of the arrows are proportional to the transition rates between each possible state combination inferred under the dependent model and averaged over 100 phylogenies (larger arrows denote higher rates; no arrows for rates of 0), when sexual systems are categorized into (A) sexually monomorphic vs. sexually dimorphic, and (B) nondioecy vs. dioecy. The number at the top of each panel denotes the number of extant species used in the analysis; percentages denote the fraction of extant species with that state combination. The size of each circle is proportional to the persistence of that state, defined as the inverse of the sum of transition rates away from it. The color of the upper half of the circles represents the ploidy level (blue, diploid; red, polyploid) and the color of the lower half, the state of the sexual system (brown, sexual monomorphism/nondioecy; green, sexual dimorphism/dioecy). *Mammillaria* is absent from (B) due to insufficient data under the dioecy–nondioecy classification.

However, the different results acquired under different categorization systems also indicate that when the total number of contrasts available is relatively low, results are easily affected by a small number of changes in the data set.

The sister-clade method also has some inherent caveats, noted before but exemplified here. The main drawback of applying sister-clade comparison techniques is that they ignore a large portion of the data and only consider clades in which contrasts in both traits appear. Analyzing a subset of the data available obviously decreases the power of the analyses, possibly missing important coevolutionary signals, thereby producing false negatives. Ignoring certain portions of the data may also lead to false positives, since taxa that are not part of any contrast may contain additional information that is inconsistent with the pattern displayed by the contrasting clades. The method is also sensitive to small perturbations in the set of taxa included in the analysis, since addition of even a single taxon may result in the omission (or inclusion) of a contrast.

Results of the analysis using Pagel's method indicated that a pattern of correlation between polyploidy and sexual system occurs in certain clades, while in others the traits seem to evolve independently of each other. The signals of correlation could reflect the false positives to which this method is prone (Maddison and FitzJohn, 2014), though we have taken some measures to address that concern. Specifically, when assessing statistical significance, we applied a parametric bootstrapping approach that accounts for the observed patterns of transition exhibited in each group, and we confirmed that several transitions in each state occur within each genus. This correction is by no means a full one for the problem, but given the dearth of other model-based methods for testing correlation in discrete characters, it seems a reasonable way forward. The lack of support for correlation in most clades might reflect low power of the method or the available data to detect subtle coevolutionary signals in these clades instead of an absence of correlated evolution. For instance, we observed that most clades in which a signal of correlation was detected had a relatively high number of transitions. It is thus possible that when the number of transitions is small, the power may be too low to detect any correlation, although to our knowledge, this has never been shown.

In addition to the technical limitations of the methods, their ability to detect correlated evolution may be affected by the patterns found in the data. The two traits may coevolve only under some evolutionary scenarios: for example, the existence of such a correlation may be affected by other life history traits such as the growth form or the type of life cycle displayed by the taxa (Vamosi et al., 2006). It is also possible that physiological or morphological changes that often follow WGD, such as greater size or drought tolerance (Maherali et al., 2009; Hodgson et al., 2010), could facilitate the invasion of novel habitats by polyploids (Ainouche et al., 2009). In cases where the ecology of these habitats favors the evolution of separate sexes, a pattern of association may evolve (Pannell et al., 2004; Ashman et al., 2013). Another important factor that might influence the coevolution of ploidy and sexual system is whether hermaphrodites self-fertilize. If they do not, e.g., due to a self-incompatibility (SI) mechanism, inbreeding avoidance would not be a strong force driving the evolution of dioecy. The interaction of polyploidy with self-incompatibility is mediated by the potential disruption of SI by polyploidy (Stone, 2002). However, this disruption depends on the type of SI; in gametophytic (but not sporophytic) SI systems, WGD almost invariably causes an immediate transition to self-compatibility (Mable, 2004), generating the possibility of selfing and hence potentially selection for other outcrossing mechanisms. It should be noted that three of the five clades in which correlated evolution between polyploidy and dioecy was detected (*Fragaria*, *Lycium*, *and Rubus*) belong to families in which the SI mode is gametophytic, while two clades (*Mammillaria* and *Leptinella*) belong to families with sporophytic SI (Mable, 2004; Igic et al., 2008). In the other clades analyzed in this study, the ratio of gametophytic to sporophytic SI systems is 7:3. For *Allocasuarina* (Casuarinaceae) and *Asparagus* (Asparagaceae), the mode of SI (if any) was not reported, while *Rumex* (Polygonaceae) was reported to be heterostylous.

The two comparative methods used in this study pursue the problem of detecting correlated evolution between discrete traits by applying distinct approaches, each with its own advantages and caveats. While the sister clade analysis approach provides global measures of the coevolutionary pattern across multiple groups, Pagel's modeling technique only allows the analysis of individual clades. On the other hand, Pagel's method is based on an evolutionary model, thus allowing for more delicate means by which associations can be detected. Certainly, our study emphasizes the need for detailed evaluation of the strengths and weaknesses of the different approaches currently employed for the detection of coevolutionary patterns.

A caveat of all analyses in this study is their over-simplification of the concept of sexual system, which requires the categorization of many distinct reproductive strategies into discrete binary classes. This simplification is essential since treating each type of sexual system as a separate state would result in high-dimension parameter space and low statistical power. This simplification, on the other hand, would necessarily lead to incomplete representation of the complexity of evolutionary scenarios in our data. For example, the sexual system assignments, as available through the Tree of Sex database, do not account for cryptic dioecy (Mayer and Charlesworth, 1991), a condition in which morphologically hermaphroditic flowers function only as male or as female, resulting in a functionally dioecious sexual system (e.g., *Lycium fremontii* and *L. exsertum*; Miller and Venable, 2003). Moreover, we did not account for intraspecific polymorphism in either polyploidy or sexual system, although it has been shown to have interesting implications in *Lycium* (Yeung et al., 2005; Blank et al., 2014; Levin et al., 2015; Miller et al., 2015). For instance, the species *L. californicum* includes distinct populations, which are usually either diploid and monomorphic or tetraploid and dimorphic (Miller et al., 2015). It is thus listed in the Tree of Sex as "hermaphroditic or gynodioecious" and categorized as nondioecious under the dioecy–nondioecy classification and as missing data under the sexual dimorphism–sexual monomorphism one. Similar intraspecific intricacies occur in *L. minimum* and *L. carolinianum*, and these correlations at the population level were overlooked here since the methods currently available for detection of correlated evolution are not capable of incorporating such data into the model.

Finally, our study highlights the importance and difficulty of examining in a phylogenetic context the association between transitions in ploidy and transitions in sexual systems, two traits whose effect on plant evolution is enormous. Obviously, an analysis of several large phylogenies, each encompassing dozens of transitions would result in more robust inferences and might reveal more delicate patterns. However, despite recent efforts (Tree of Sex Consortium, 2014; Renner, 2014), sexual system data at the species level are sparse, currently preventing the application of sophisticated

comparative methods that require trait data at the species level on large phylogenies. Moreover, analyzing large phylogenies would entail the consideration of more ancient WGD events and, consequently, the possibility of diploidization. An ultimate solution would be to track transitions in ploidy through time, thereby allowing the assignment of certain parts of the phylogeny to polyploidy or functional diploidy. This, in turn, would allow the investigation of the interplay of ploidy and sexual system, as well as other key traits, using larger phylogenies.

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