Where do monomorphic sexual systems fit in the evolution of dioecy? Insights from the largest family of angiosperms

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Summary

• A range of hypothesized evolutionary pathways has been proposed for describing the evolution of dioecy. However, the evolutionary links between other sexual systems not directly involved in dioecy evolution remain largely unexplored, and hence, a comprehensive picture of evolutionary transitions between sexual systems is still lacking.
• Here, we explored the diversity and evolution of sexual systems in Asteraceae, the largest family of flowering plants, where almost all sexual systems are present. We used a phylogenetic approach to build a model of evolutionary transitions between sexual systems.
• The best model involved nine transitions, including those from hermaphroditism to andromonoecy, gynomonoecy and gynodioecy, those from gynomonoecy to monoecy and trimonoecy, two transitions to dioecy –one through gynodioecy and the other through monoecy – and reversals from monoecy to gynomonoecy and from gynomonoecy to hermaphroditism.
• Our reconstruction of the evolution of sexual systems in Asteraceae provided, for the first time, a joint view of the evolutionary transitions between seven sexual systems, unveiling the evolutionary links between monomorphic sexual systems. A pathway from hermaphroditism to monoecy through gynomonoecy, instead of from andromonoecy, was highly supported, which was consistent with a gradient of floral gender specialization.

Introduction

Most flowering plants produce exclusively bisexual or hermaphroditic flowers, bearing female and male organs on the same flower (Yampolsky & Yampolsky, 1922). However, a substantial proportion of species arrange their female and male organs on different flowers and/or even on different individuals (Yampolsky & Yampolsky, 1922; Barrett, 2002; Charlesworth, 2006). In line with many authors (Ramírez, 2005; Obbard *et al.*, 2006; Zhang *et al.*, 2006; Case *et al.*, 2008; Pannell *et al.*, 2008; Torices & Anderberg, 2009), we refer to these different arrangements of sexual organs on different flowers and/or individuals as sexual systems. In the so-called monomorphic sexual systems (Bawa & Beach, 1981), species bear bisexual, female and/or male unisexual flowers on the same individuals, such as in monoecy, gyno-

monoecy, andromonoecy and trimonoecy (Fig. 1a). Other species have dimorphic sexual systems, that is, unisexual individuals (Bawa & Beach, 1981), such as in dioecy, gynodioecy and androdioecy (Fig. 1a). This variation has important functional consequences for mating behaviour and plant fitness, and hence to understand its origin and maintenance is an important challenge for plant biologists (Richards, 1997; Barrett, 2002; Charlesworth, 2006).

Strong empirical evidence indicates that production of only bisexual flowers is the ancestral condition in angiosperms (Richards, 1997; Doyle, 1998; Endress, 2001). Hence, all this diversity in the arrangement of bisexual and unisexual flowers has repeatedly evolved from a bisexual-flowered ancestor. Nevertheless, we do not know if, for instance, all sexual systems have evolved directly from the ancestral bisexual condition, or if some of them have arisen

(a) Main evolutionary pathways to dioecy
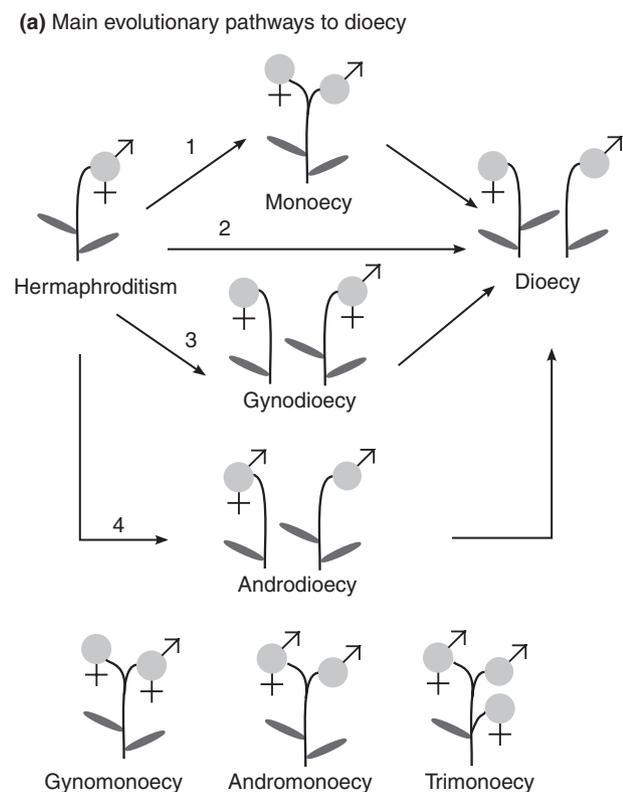
(b) Expectations of the sexual selection hypothesis
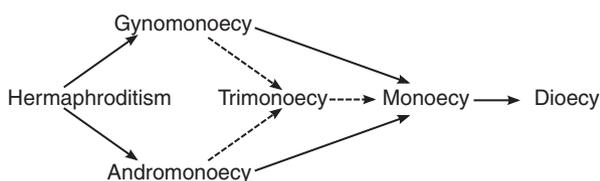
Fig. 1 Evolutionary pathways to dioecy. (a) Evolutionary pathways to dioecy in flowering plants and a pictorial description of plant sexual systems. (b) Evolutionary transitions between sexual systems expected according to the sexual selection hypothesis for the evolution of dioecy. Flowers and their gender are depicted as light grey circles. Four main pathways have been proposed to explain the evolution of dioecy: (1) via monoecy; (2) directly from hermaphroditism (including both heterodichogamous or duodichogamous hermaphrodites, and dimorphic heterostylous hermaphrodites); (3) via gynodioecy, and (4) via androdioecy. Gynomonoecy, andromonoecy and trimonoecy have not been explicitly included in any pathway to dioecy. Androdioecy, male and bisexual flowers on different plants; andromonoecy, male and bisexual flowers on the same plant; dioecy, male and female flowers on different plants; gynodioecy, female and bisexual flowers on different plants; gynomonoecy, female and bisexual flowers on the same plant; hermaphroditism, plants with hermaphroditic, or bisexual, flowers; monoecy, unisexual male and female flowers on the same plant; trimonoecy, female, male and bisexual flowers on the same plant.

floral and individual sexual specialization, as well as information for more in-depth studies of the ecological basis of selection (Barrett, 2008).

Many researchers have directed their investigations towards disentangling what evolutionary transitions have given rise to dioecy (Darwin, 1877; Bawa, 1980; Lloyd, 1982; Weiblen *et al.*, 2000; Renner & Won, 2001; Gleiser & Verdú, 2005; Case *et al.*, 2008). Thus, a variety of hypothesized evolutionary pathways have been proposed for the evolution of dioecy from hermaphroditism (Ross, 1980, 1982; reviewed in Webb, 1999) (Fig. 1a). Not all these pathways have received the same theoretical attention or empirical support. Most research has focused on pathways involving gynodioecy (Charlesworth & Charlesworth, 1978a; Bailey *et al.*, 2003; Jacobs & Wade, 2003) or monoecy (Charlesworth & Charlesworth, 1978b; Bawa, 1980; Lloyd, 1980).

By contrast, the relationships between other sexual systems not directly involved in dioecy evolution remain largely unexplored; for example, from which sexual systems have originated, and whether other sexual systems have evolved from them. Thus, monoecy has traditionally been considered as originating from andromonoecy or gynomonoecy (Charlesworth & Charlesworth, 1978b; Willson, 1979; Bawa & Beach, 1981; Bertin, 1982). Although several factors could fuel the evolution of nonhermaphroditic sexual systems (e.g. herbivory: Ashman, 2002; Bertin *et al.*, 2010), most attention has focused on the sexual selection hypothesis of dioecy evolution (Charlesworth & Charlesworth, 1978b; Willson, 1979; Bawa & Beach, 1981; Bertin, 1982) (Fig. 1b). According to this hypothesis, avoidance of interference between female and male floral functions has driven sexual specialization of flowers into pollen donors and receivers. This hypothesis leads to the expectation that, starting with an ancestor of bisexual flowers, unisexual flowers will evolve on the same individual, and finally unisexual individuals will be selected (Fig. 1b). Nevertheless, a theoretical sex allocation model has suggested that the gynomonoecious pathway is improbable, since it requires unrealistically high amounts of seed production in female flowers (de Jong *et al.*, 2008).

Empirical support for evolutionary pathways between sexual systems has traditionally come from studies at the species level (Webb, 1999) or, more recently, from comparative studies of small families or clades (Weller & Sakai, 1999). In addition, phylogenetic reconstructions of transitions between sexual systems have been performed for an increasing number of lineages (Renner & Won, 2001; Weiblen, 2004; Gleiser & Verdú, 2005; Renner *et al.*, 2007; Case *et al.*, 2008; Torices & Anderberg, 2009). Although valuable, these studies yield only a piecemeal picture of the evolution of sexual systems (Weller & Sakai, 1999). This is because most plant families have a very limited – two or three – number of nonhermaphroditic

from other nonhermaphroditic sexual systems. The clarification of these evolutionary transitions between sexual systems can provide an insight into the ecological drivers of

sexual systems. Overcoming these limitations of available studies requires data sets that include a wider portion of the existing sexual systems (Weiblen *et al.*, 2000). In this way, a more detailed view of the evolutionary links between less common sexual systems, and a more sound comparison of the different pathways of evolution of dioecy are possible. One way to achieve this is to perform analyses of large clades by pooling many plant families (Weiblen *et al.*, 2000; Zhang *et al.*, 2006). However, these large-scale approaches are usually associated with a low taxon sampling. A complementary method is to study lineages that show high diversity in sexual systems (Weeks *et al.*, 2009), in which better taxon sampling can be achieved, thus improving the inferences of evolutionary processes (Gittleman *et al.*, 2004).

Here, we explored the diversity and evolution of sexual systems in Asteraceae, the largest Angiosperm family. We used a phylogenetic approach to model the evolutionary pathways between sexual systems. Thus, we have inferred what evolutionary transitions have probably occurred between sexual systems and the rate of change of each transition. We have chosen Asteraceae, because it shows one of the broadest ranges of sexual systems with Amaranthaceae and Poaceae (M. Méndez, unpublished). Aside from the insights provided by Lloyd (1972a,b, 1975a,b) using a nonphylogenetic approach, previous work has studied transitions between sexual systems only in small subsets of this family (Swenson & Bremer, 1997 for the genus *Abrotanella*; Watson *et al.*, 2002 for the Subtribe Artemisiinae; Torices & Anderberg, 2009 for the tribe Inuleae). In particular, we address two sets of related questions about the patterns of evolution of sexual systems. Our first set of questions focuses on the role of monomorphic sexual systems: is hermaphroditism ancestral; and has monoecy evolved from gynomonoecy and andromonoecy? Our second set of questions refers to the main pathways to dioecy: what sexual systems have given rise to dioecy; and what is the most common evolutionary pathway leading to dioecy? Finally, we discuss which factors might have influenced in the observed diversity of sexual systems and the pattern of evolutionary transitions between monomorphic sexual systems in Asteraceae.

Materials and Methods

Sexual system data

Sexual systems were classified into seven categories (Cruden & Lloyd, 1995): hermaphroditism, monoecy, andromonoecy, gynomonoecy, trimonoecy, dioecy and gynodioecy (Fig. 1a). Other reproductive strategies also referred to as sexual systems in some contexts but not involving sexual specialization at the flower or plant levels, such as heterostyly or dichogamy, are not relevant for this study and were not considered. Some authors (Richards, 1997) include within hermaphroditism all those cases in which both male

and female flowers are born on the same individual. Our terminology follows Darwin (1877) and uses hermaphroditism in a restricted sense to mean only those species bearing exclusively bisexual flowers. This is justified because our objectives require to clearly separate all these strategies diverging in gamete packaging (Lloyd, 1979) despite producing cosexual individuals, that is, andromonoecy, gynomonoecy, trimonoecy and monoecy.

We collected information on sexual systems for 1597 Asteraceae genera, using the published literature including original taxonomic descriptions, floras and surveys of sex expression (Tutin *et al.*, 1976; Bremer, 1994; Herman *et al.*, 2000; Nesom, 2000; Bayer *et al.*, 2002; Baldwin, 2003; Anderberg *et al.*, 2007). This data set is available at Dryad repository (http://dx.doi.org/10.5061/dryad.7960). Genera are suitable study units for our purposes because sexual systems in Asteraceae are, with a few exceptions, constant within genera (Bremer, 1994; Anderberg *et al.*, 2007). Only 151 genera had more than one sexual system (see the following section for coding of these genera). We did not consider temporal and spatial intrafloral variation, as no information is available for the whole family.

Phylogenetic data

We reconstructed the sexual system evolution in Asteraceae by using a supertree assembled for the family (Funk *et al.*, 2005). We considered this phylogeny as suitable for our purposes because it has a good taxonomic coverage of the family: it includes 405 terminal taxa, representing one-quarter of all extant genera of Asteraceae, and taxa from all tribes and all sexual systems are represented. Two outgroups were included in the supertree: Goodeniaceae and Calyceraceae. Calyceraceae was coded as hermaphroditic and andromonoecious because of the presence of functionally male central disk florets in *Acicarpha* (Hellwig, 2007). Goodeniaceae has a hermaphroditic sexual system (Carolin, 2007). Four *Senecio* species were pruned from the supertree because sexual system information was uncertain. Sixty-one genera having more than one sexual system were included in the supertree. The occurrence of more than one state in a terminal taxon is problematic for inferring ancestral states and we therefore split those genera having more than one sexual system into as many separate clades as sexual systems present (Nixon & Davis, 1991; see Weiblen *et al.*, 2000 for application of this procedure to sexual system reconstruction). Treatment of polymorphic genera by this procedure expanded the supertree to 475 branch tips. All trees and sexual system data used in the following analyses are available at Dryad repository (http://dx.doi.org/10.5061/dryad.7960).

The frequency of taxa having different sexual systems in the phylogenetic data set (i.e. 405 genera included in Funk *et al.*, 2005) differed significantly from that in the full data set (i.e. 1597 genera) irrespective of the codification of sexual

systems (polymorphism kept, χ^2 = 19.64, df = 5, P = 0.001; polymorphism resolved, χ^2 = 72.09, df = 5, P = 0.0000; Table 1). Hermaphroditism and monoecy were underrepresented, whereas the combination of hermaphroditism and gynomonoecy was overrepresented, in the phylogenetic data set. Notwithstanding, these over- or underrepresentations were quantitatively slight and all sexual systems occurring in Asteraceae were represented in our phylogenetic data set, as well as the main combinations of sexual systems for genera with several sexual systems.

The conditions needed for a reconstruction of ancestral characters regarding branch lengths, tree topologies and character state frequencies on the tips (Oakley, 2003; Nosil & Mooers, 2005; Goldberg & Igic, 2008) were addressed as follows. First, although the supertree utilized is a consensus tree and does not have branch lengths (Funk *et al.*, 2005), we used a new version with time-calibrated branch lengths (Torices, 2010; available at http://dx.doi.org/10.5061/dryad.7960). The use of different branch lengths (equal branch lengths or setting under a birth and death model) only influenced the total number of transitions between sexual systems in the maximum likelihood (ML) reconstructions (Torices, 2010), whereas Bayesian reconstructions were very similar, irrespective of branch lengths used (Supporting Information, Table S1).

Secondly, polytomies in the original supertree were randomly resolved to avoid difficulties in inferring ancestral states (Maddison & Maddison, 2009). We generated a set of 500 dichotomous trees and conducted the analyses on them. We accounted for uncertainty in the tree topology by reconstructing character evolution on this set of randomly dichotomous trees and checking whether different tree topologies yielded different character evolution. Branch lengths were previously rescaled to one with proportions maintained.

Thirdly, the differences between the frequencies of sexual systems in our phylogenetic dataset with the full lineage might produce a biased estimation of transition rates toward the biased states under unconstrained ML estimations because the stationary frequencies under a Markov model of discrete character evolution are assumed to be equal *a priori*. Thus, transition rates estimated may be biased towards the more common state on the tips of the phylogeny (Nosil & Mooers, 2005; Goldberg & Igic, 2008). To avoid this caveat it would be necessary to incorporate the diversification effects of character states (Goldberg & Igic, 2008). Unfortunately, the analytical tools such as BiSSE (Maddison *et al.*, 2007) require that all species be included in the tree and that the state for each species be known, which is not availabe for the *c.* 25 000 species of the Asteraceae family.

Modelling the evolutionary transitions between sexual systems in Asteraceae

Our modelling of the evolutionary transitions between sexual systems in Asteraceae proceeded in four steps. First, we estimated the most likely sexual system at the root of Asteraceae. Secondly, we mapped sexual systems on the phylogeny. Thirdly, we fitted statistical models of evolutionary transitions using the information provided by the two previous steps (ancestral sexual system estimation and sexual system mapping), considering also empirical data about sexual system transitions in Asteraceae. Finally, we selected the fittest model and calculated a model-averaged estimate of each transition rate.

Ancestral sexual system estimation To examine the robustness of our evolutionary inference (Ronquist, 2004), and following recent recommendations (Ekman *et al.*, 2008;

Table 1 Frequency (and percentage) of genera with different sexual systems in Asteraceae

| Sexual system | Asteraceae (%) | | | | Angiosperms |
	Single	In combination	Original Supertree	Supertree without polymorphisms	%
Hermaphroditism	779 (48.78)	97 (6.07)	173 (43.14)	221 (46.53)	72.0
Gynomonoecy	489 (30.62)	134 (8.39)	125 (31.17)	180 (37.89)	2.8
Monoecy	139 (8.70)	49 (3.07)	25 (6.23)	36 (7.58)	5.0
Dioecy	25 (1.57)	8 (0.50)	8 (2.00)	11 (2.32)	4.0
Andromonoecy	8 (0.50)	15 (0.94)	3 (0.75)	12 (2.53)	1.7
Trimonoecy	4 (0.25)	13 (0.81)	–	10 (2.10)	–
Gynodioecy	2 (0.13)	4 (0.25)	1 (0.25)	5 (1.05)	7.0
Several present	151 (9.46)		66 (16.46)	–	–

'Single' refers to genera having exclusively the indicated sexual system; 'In combination' shows the genera in which that sexual system is present in combination with any of the others. 'Original supertree' and 'Supertree without polymorphism' show the genera in each supertree having those sexual systems. The last column shows the percentage representation of each sexual system in angiosperms (Yampolsky & Yampolsky, 1922; Richards, 1997). Asteraceae data set (*n* = 1597); original supertree data set (*n* = 401); supertree without polymorphism data set (*n* = 475).

Xiang & Thomas, 2008), the ancestral sexual system was inferred using three different approaches: maximum parsimony (MP), ML and Bayesian analyses (BA).

We implemented unordered parsimony reconstruction with Mesquite 2.6 software (Maddison & Maddison, 2009) on the original supertree (Funk et al., 2005) and on a set of 500 trees (see the 'Phylogenetic data' section). We assessed whether elimination of polymorphisms yielded different ancestral states in the MP reconstruction compared with the original supertree. The software for ML and BA did not allow polymorphisms in terminal taxa; therefore we used only the trees where polymorphisms were resolved (see the 'Phylogenetic data' section). For ML reconstruction, we used the same software as for MP reconstruction. We selected the Mk1 model ('Markov k-state 1 parameter model'), that is, a k-state generalization of the Jukes–Cantor model, which corresponds to Lewis's (2001) Mk model (Maddison & Maddison, 2006).

The BA reconstruction under continuous-time Markov models was implemented with SIMMAP 1.0 Beta 2.3.2 software (Huelsenbeck & Bollback, 2001; Bollback, 2006). BA requires the use of priors (Schultz & Churchill, 1999). SIMMAP offers two priors, the overall rate and the two-state bias parameter prior (Bollback, 2006). The two-state bias parameter cannot be specified for multistate characters (Bollback, 2006), and hence we only set the overall rate prior. The choice of the most reasonable evolutionary rates a priori has to be made subjectively by each researcher, by using parameters that are independent of the data or the model (Schultz & Churchill, 1999). To assess whether the particular form of the prior dominated the posterior results (Schultz & Churchill, 1999; Pagel et al., 2004), we explored three different priors to obtain the posterior distribution. Lacking information on how sexual systems have evolved in Asteraceae, three priors were set, describing a gamma distribution from 'lower' $E(T) = 1.00$, $SD(T) = 1.00$, through 'medium' $E(T) = 1.50$, $SD(T) = 0.87$ to 'higher' values $E(T) = 5.00$, $SD(T) = 2.24$. The gamma distribution was made discrete using 60 categories. The posterior probability of each gamma category defined by the prior was then calculated and a stochastic draw was made from this distribution. The rate value, for the sampled category, was used as multiplier of branch lengths (Huelsenbeck et al., 2003). The three prior distributions yielded the following posterior mean rate values: 5.09, 4.50 and 12.28. The differences between the posterior mean rates are produced because they are calculated combining the gamma prior with the character observations (Huelsenbeck et al., 2003). The tree length for the higher prior distribution was more than twice as large as that for the other two priors, and therefore we could expect more than double the number of changes for this prior than for the other two priors.

We studied the marginal posterior probability of states in the node that include all Asteraceae.

Sexual system mapping We mapped transitions between sexual systems by means of the MP, ML and BA reconstructions on a set of 500 trees (see the 'Phylogenetic data' section). The MP and ML reconstructions were implemented in Mesquite 2.6 software (Maddison & Maddison, 2009). Two hundred mappings were sampled per tree in the MP analysis to achieve the same sample size as in the BA reconstruction (100 000 mappings), and one mapping per tree in the ML analysis because this analysis does not allow for multiple mappings of character reconstructions (Maddison & Maddison, 2006). Mesquite provides the mean, minimum and maximum number of changes of each transition over all inferred mappings. The BA character mapping technique (Huelsenbeck et al., 2003) was implemented in the program SIMMAP 1.0 Beta 2.3.2 (Bollback, 2006; see Renner et al., 2007 and Torices & Anderberg, 2009 for a recent use of this methodology in sexual system evolution). Twenty maps or realizations were sampled from each tree and 10 realizations were sampled from the prior distribution over three sets of morphological priors to test the influence on the results. The three priors on the rate parameter were set as described earlier. We reported the number of transitions as the mean ± SD, mode and range. The mode was a good estimate of the number of transitions and was always very close to the mean.

Models of evolutionary transitions We assessed which evolutionary pathways fitted the data better, assuming that transitions between sexual systems do not have to be equally likely, as is assumed by the MP, ML and BA methods of trait mapping. Thus, we modelled the evolutionary transitions between sexual systems using the Bayesian Markov chain Monte Carlo (MCMC) approach (Pagel & Meade, 2006) as implemented in the BayesTraits software version 1.0 (available at website http://www.evolution.rdg.ac.uk). This method is based on a continuous-time Markov model, which models the transitions of discrete characters between states by estimating a rate parameter for each different transition. In our study, for a seven-state trait such as sexual system, there are 42 possible parameters q_{ij}, which are the instantaneous rates of change from state i to j, assuming that, instantaneously, only a single change may occur in one trait.

We followed the following steps to build the best evolutionary model of transitions between sexual systems in Asteraceae. First, we used as initial models (hypotheses) the evolutionary transitions among sexual systems obtained from the MP, ML and BA reconstructions, retaining only those transitions estimated in at least 95% of the simulations for BA, and those transitions with more than one mean change for MP and ML (Fig. 2). Secondly, we tested

(a) Bayesian mapping: MarLik = −538.283 ± 0.089; BIC = 1144.362

(b) Maximum likelihood mapping: MarLik = −550.259 ± 0.084; BIC = 1155.988

(c) Maximum parsimony mapping: MarLik = −531.415 ± 0.058; BIC = 1136.790
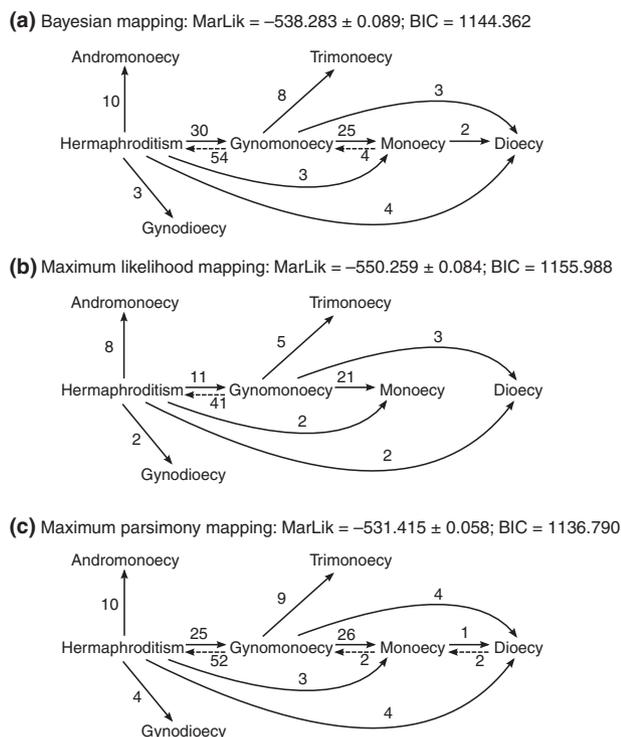
Fig. 2 Evolutionary transitions between sexual systems in Asteraceae obtained from sexual system mapping. (a) Absolute number of changes is indicated as the modal value for each transition from Bayesian character reconstruction. Transitions represented were retrieved in at least 95% of all simulations (Table 3) using a flat prior ($E(T)$ = 0.50 and $SD(T)$ = 0.50) and 100 000 realizations. (b) Absolute number of changes for maximum likelihood reconstructions. (c) Absolute number of changes for maximum parsimony reconstruction. (b) and (c) show transitions with more than one mean change. Dashed arrows indicate reversals. For each model, the marginal likelihood (MarLik) and the Bayesian information criterion (BIC) is shown.

which of these three empirical hypotheses obtained from sexual system mapping, BA, MP or ML, once simplified through the first step, was a better model of sexual system evolution given our data set and this supertree. Thirdly, we further refined the MP and BA mappings, because the ML mapping model clearly did not fit the data as well as the MP and BA mappings (ΔBIC > 10; Fig. 2). We did this by testing the influence of three evolutionary transitions inferred by MP and BA mapping analyses, but they did not have theoretical support or had little empirical support (Fig. 2): (i) direct transition from hermaphroditism to dioecy (HtoD); (ii) direct transition from hermaphroditism to monoecy (HtoM); and (iii) direct transition from gyno-monoecy to dioecy (GmtoD). Fourthly, we asked whether the transition from gynodioecy to dioecy (GdtoD) should be included in the final model of evolutionary transitions to dioecy, although it was not retrieved by any of the character-mapping reconstructions (Fig. 2). The reason was that this evolutionary transition has empirical support in

Asteraceae, because evidence suggests that dioecy has evolved via gynodioecy from hermaphroditism at least in *Cirsium* (Delannay, 1978, 1979; Lloyd & Myall, 1976).

In order to test the likelihood of transitions with no or little empirical support (HtoD, HtoM and GmtoD), we built additional models in which these transitions were removed (see a pictorial description of each model in Fig. S1). When more than one of these modifications improved the model, we tested if the removal of multiple transitions (e.g. HtoD and HtoM) at once produced a better model. We built two different models to explore whether the GdtoD transition should be included, allowing or removing a direct transition from hermaphroditism to dioecy (models 'MP/BA-GdtoD-1' and 'MP/BA-GdtoD-2', respectively; Fig. S1). The hypothesis was that the evolutionary transition from hermaphroditism to dioecy could actually be a transition through a gynodioecious intermediate (see Weiblen *et al.*, 2000).

Each model was fitted by fixing the ancestral node for the whole family to hermaphroditism, which was the most likely sexual system at the root of the family (see the Results section). We used the same set of trees as in previous analyses (see the Phylogenetic data section). After testing several hyperprior distributions (results not shown), we seeded the mean of the distribution of rate coefficients, drawing from an exponential (0–5) hyperprior distribution (see Pagel & Meade, 2006 for further information on hyperprior distributions) since it was the only one that produced an acceptance rate between 20 and 40%, as the BayesTraits manual suggests. Run lengths and burn-in periods were started at 6×10^6 and 10^5 generations, respectively, and both were increased until the effective sample size for all parameters exceeded 50 (estimated in Tracer v1.4 software; available at website http://beast.bio.ed.ac.uk/Tracer). Marginal likelihood, the criterion of goodness-of-fit for the model (Raftery, 1996), was estimated using the method proposed by Newton & Raftery (1994) with the modifications proposed by Suchard *et al.* (2001), which is implemented in Tracer v1.4 software. SE for marginal likelihood was estimated using 1000 bootstrap replicates.

Overall, we compared 15 models (Figs 2, S1). To avoid an inordinate number of pairwise model comparisons, we selected models based on Bayesian information criterion (BIC). BIC has some desirable properties: it compares all candidate models at once (Posada, 2009); it does not require nested alternatives (Bolker, 2008); and a collection of BIC statistics contains the same information as a collection of pairwise Bayes factors but are easier to interpret by visual inspection (Posada & Buckley, 2004; Bolker, 2008). In addition, given equal priors for all competing models, choosing the model with the smallest BIC is equivalent to selecting the model with the maximum posterior probability (Bolker, 2008; Posada, 2009). Nevertheless, we also explored that BIC results agreed with results based on AIC

and AICc (Bolker, 2008). Models with BICs < 2 apart (ΔBIC < 2) are more or less equivalent; those with BICs 4–7 apart are clearly distinguishable; and models with BICs > 10 apart are definitely different (Bolker, 2008). Eventually, BIC allows the assessment of model selection uncertainty, as well as the estimation of model parameters using all available models (model-averaged inference or multimodel inference) (Posada & Buckley, 2004; Sullivan & Joyce, 2005; Bolker, 2008; Posada, 2009). Hence, we accounted for the model selection uncertainty with the BIC by estimating the approximate posterior probabilities (PPs) of each model to calculate a model-averaged estimate of transition rates as described by Raftery (1996) and Bolker (2008).

Results

Sexual system diversity in Asteraceae and its ancestral state

Over 50% of all Asteraceae genera (n = 1597) showed sexual systems other than hermaphroditism (Table 1) and all the major sexual systems were represented in Asteraceae except androdioecy (Table 1). Hermaphroditism was retrieved as the ancestral sexual system, irrespective of the reconstruction method and the character scoring used (Table 2).

Transitions between sexual systems provided by character mapping

There was a broad agreement across MP, ML and BA methods in the transitions between sexual systems. The transition from hermaphroditism to gynomonoecy and the

from gynomonoecy to monoecy, as well as the direct transition from hermaphroditism to monoecy, was retrieved in all reconstruction methods (Table 3; Fig. 2). Other transitions retrieved were from a hermaphroditic ancestor to andromonoecy and from gynomonoecy to trimonoecy (Table 3; Fig. 2). Reversals were found from gynomonoecy to hermaphroditism and from monoecy to gynomonoecy, except for the ML analysis (Table 3; Fig. 2).

Hermaphroditism, gynomonoecy and monoecy gave rise to dioecy in all mapping methods, except for monoecy in the ML analysis (Table 3, Fig. 2). No transition from gynodioecy to dioecy was inferred (Table 3, Fig. 2). Reversals from dioecy to monoecy were reconstructed only using MP analysis (Table 3; Fig. 2).

The total number of changes between sexual systems was dependent on the method of reconstruction, ranging from 96.53 in the ML analysis, through 148.89 in the MP, to 161.33 ± 2.36 in the BA (Table 3). Most changes occurred between hermaphroditism and gynomonoecy and from gynomonoecy to monoecy (Table 3; Fig. 2).

Choosing a model of evolutionary transitions

The model for the evolution of sexual systems obtained from MP mappings fitted the data better than those from the ML (ΔBIC = 19.20; Fig. 2) and the BA mappings (ΔBIC = 7.57; Fig. 2). Nevertheless, some of the modifications performed over the MP and BA models reached lower BIC values (Table 4). Thus, the evolutionary model was improved by removing transitions without biological support on both reference models, that is, from hermaphroditism to monoecy and from gynomonoecy to dioecy (Table 4; Fig. 3). By contrast, removal of transitions from hermaphroditism to dioecy produced contradictory results because this transition was not allowed in the best model, whereas in the second and the third best models, changes from hermaphroditism to dioecy were allowed (Table 4; Fig. 3). Nevertheless, the rate of change from hermaphroditism to dioecy was always very low (Fig. 3). Furthermore, the inclusion of a transition between gynodioecy and dioecy, which had not been reconstructed by any of the mapping methods, remarkably improved the evolutionary model of transitions (Table 4; Fig. 3a).

The best model included nine transitions (PP = 0.87; Table 4; Fig. 3a), including those from hermaphroditism to andromonoecy, gynomonoecy and gynodioecy, those from gynomonoecy to monoecy and trimonoecy, two transitions to dioecy – one through gynodioecy and the other through monoecy – and reversals from monoecy to gynomonoecy and from gynomonoecy to hermaphroditism. Nevertheless, the model-averaged estimate of the first five best models (PP > 0.99) raised the number of transitions to 11, adding the reversal from dioecy to monoecy, and the direct transition from hermaphroditism to dioecy (Fig. 3f).

Table 2 Estimation of the ancestral sexual system in Asteraceae using maximum parsimony, maximum likelihood and Bayesian methods

Method	Tree	Ancestral sexual system
Parsimony	Original	Hermaphroditism
Parsimony	Randomly resolved	Hermaphroditism in all trees
Likelihood	Randomly resolved	Hermaphroditism in all trees[a]
Bayesian	Randomly resolved	Hermaphroditism[b]

'Original', when Funk et al. (2005) supertree was used as phylogenetic hypothesis without any modification. 'Randomly resolved', when a set of 500 randomly resolved polytomies of the 'original' tree were used as phylogenetic hypothesis.
[a]Hermaphroditism was estimated as the best state at the root of all (500) trees analysed. The relative averaged probability for hermaphroditism at the root was 0.995.
[b]Ancestral posterior probability was 1.00 for hermaphroditism at the studied node and irrespective of the rate parameter prior distribution. We used three sets of prior distributions ($E(T)$ = 0.50, SD(T) = 0.50; $E(T)$ = 1.50, SD(T) = 0.87; $E(T)$ = 5.00, SD(T) = 2.24) and the results were identical for all of them.

Table 3 Number of estimated changes among sexual systems in Asteraceae (mean ± SD for Bayesian analysis (BA), and mean (range) for maximum likelihood (ML) and maximum parsimony (MP) analyses)

From\To	Method	Hermaphroditism	Andromonoecy	Gynomonoecy	Monoecy	Dioecy	Gynodioecy	Trimonoecy
Hermaphroditism	BA		**10.14 ± 0.75****	**30.35 ± 2.88****	2.85 ± 1.10**	**4.21 ± 0.99****	3.42 ± 0.98**	0.94 ± 0.77
	ML		**8.00 (8–8)**	**11.00 (11–11)**	**2.00 (2–2)**	2.04 (0–3)	**2.35 (1–3)**	0
	MP		10.50 (10–12)	24.96 (15–37)	2.55 (2–7)	4.26 (2–6)	3.97 (1–5)	0.23 (0–2)
Andromonoecy	BA	1.82 ± 0.99		0.84 ± 0.61	0.03 ± 0.17†	0.01 ± 0.09‡	0.00 ± 0.05‡	0.23 ± 0.42
	ML	0		0	0	0	0	0
	MP	0.52 (0–2)		0.50 (0–1)	0	0	0	0.11 (0–1)
Gynomonoecy	BA	**53.57 ± 2.37****	0.72 ± 0.68		**25.24 ± 1.30****	3.21 ± 0.84**	1.06 ± 0.75	**8.21 ± 0.96****
	ML	**41.17 (37–44)**	0.48 (0–1)		**20.97 (19–22)**	**3.00 (3–3)**	0	**5.46 (5–6)**
	MP	**52.19 (40–64)**	0.87 (0–2)		**25.81 (20–30)**	3.96 (2–6)	0.82 (0–2)	**9.21 (7–10)**
Monoecy	BA	0.99 ± 0.75	0.01 ± 0.10‡	**3.79 ± 0.96****		2.03 ± 0.84*	0.01 ± 0.08‡	0.71 ± 0.46
	ML	0	0	0		0	0	0
	MP	0.26 (0–4)	0	**2.41 (1–7)**		1.06 (0–3)	0	0.45 (0–1)
Dioecy	BA	1.08 ± 0.83	0.01 ± 0.08‡	0.13 ± 0.34	1.20 ± 0.85		0.60 ± 0.65	0.00 ± 0.07‡
	ML	0	0	0	0		0	0
	MP	0.73 (0–3)	0	0.50 (0–1)	1.53 (0–4)		0.22 (0.2)	0
Gynodioecy	BA	0.70 ± 0.78	0.01 ± 0.08‡	0.38 ± 0.56	0.03 ± 0.17†	0.61 ± 0.65		0.00 ± 0.06‡
	ML	0	0	0	0	0		0
	MP	0.13 (0–2)	0	0.20 (0–2)	0	0.22 (0–2)		0
Trimonoecy	BA	0.74 ± 0.76	0.28 ± 0.45	0.85 ± 0.74	0.33 ± 0.49	0.01 ± 0.09‡	0.00 ± 0.05‡	
	ML	0	0	0	0	0	0	
	MP	0.23 (0–2)	0.11 (0–1)	0	0.27 (0–1)	0	0	

n = 100.000 except for ML character mapping, for which n = 500. The BA mapping was rather insensitive to the prior utilized (Table S1). Here, we show the results setting the rate parameter prior distribution to $E(T)$ = 0.50, $SD(T)$ = 0.50. Values in bold indicate that the transition was retrieved in all mappings. For BA, the following coding is utilized: transitions retrieved in at least 99% of the simulations (**), in at least 95% of the simulations (*), in < 5% of the simulations (†), in < 1% of the simulations (‡).

Discussion

Sexual system diversity in Asteraceae and its ancestral state

Our survey of sexual systems in Asteraceae revealed the presence of all major sexual systems, except for androdioecy. Hermaphroditism was the most frequent sexual system, but was less frequent than in the angiosperms as a whole (Yampolsky & Yampolsky, 1922), or in monocotyledons (Weiblen et al., 2000). The second most common sexual system in this family was an uncommon sexual system for the whole flowering plants, gynomonoecy (Table 1; Yampolsky & Yampolsky, 1922). Cronquist (1955) hypothesized that the Asteraceae ancestor was a gynomonoecious herb. However, our results agreed with Bremer's (1994) hypothesis that this family had a hermaphroditic ancestor.

Transitions to monoecy and other monomorphic sexual systems

Our model for the evolution of sexual systems in Asteraceae provided, for the first time, a joint view of the evolutionary transitions between seven sexual systems. In this section, we discuss the transitions to monoecy and other monomorphic sexual systems. A pathway from hermaphroditism to monoecy through gynomonoecy was highly supported (Fig. 3f) and concentrated most changes between sexual systems (Fig. 2). In agreement with the sexual selection hypothesis, a first step involved the transition from hermaphroditism to gynomonoecy by specializing some flowers into female function. In a second step, some gynomonoecious lineages evolved to monoecy by specializing the remaining bisexual flowers into male unisexual flowers. The origin of andromonoecy from hermaphroditism and trimonoecy – a minor sexual system rarely considered when addressing the evolution of nonhermaphroditic sexual systems – from gynomonoecy also fits the expectations of the sexual selection hypothesis.

Although derivation of andromonoecy from hermaphroditism supports Bertin's (1982) hypothesis, and derivation of trimonoecy from gynomonoecy fitted our expectations (Fig. 1b), our results depart from the predictions of a recent theoretical sex allocation model for the evolution of monoecy (de Jong et al., 2008). First, this model forecasts that the most likely pathway to monoecy from hermaphroditism should occur via andromonoecy and not via gynomonoecy since the latter requires unrealistically high amounts of seed production in female flowers (de Jong et al., 2008). Secondly, de Jong et al. (2008) proposed that trimonoecy is never an evolutionary stable strategy for plants. The disagreements between our results and the predictions of the theoretical sex allocation model (de Jong et al., 2008) might

Table 4 Model performance of evolutionary transitions between sexual systems in Asteraceae

Model[a]	k	MarLik	BIC	ΔBIC	PP	Cum (PP)
BA – GdtoD – 2	9	−529.733 ± 0.039	1114.936	0.000	0.8734	0.8734
MP – GdtoD – 1	11	−526.373 ± 0.048	1120.542	5.607	0.0529	0.9692
MP – HtoM & GmtoD	10	−529.665 ± 0.054	1120.963	6.027	0.0429	0.9162
MP – GdtoD – 2	10	−530.502 ± 0.072	1122.637	7.701	0.0186	0.9878
BA – GdtoD – 1	10	−531.079 ± 0.057	1123.791	8.855	0.0104	0.9982
MP – HtoM	11	−530.018 ± 0.104	1127.832	12.896	0.0014	0.9996
BA – HtoM & GmtoD	9	−537.448 ± 0.099	1130.366	15.430	0.0004	1.0000
MP	12	−531.415 ± 0.058	1136.790	21.854	0.0000	1.0000
MP – GmtoD	11	−534.527 ± 0.046	1136.850	21.915	0.0000	1.0000
BA – GmtoD	10	−538.158 ± 0.103	1137.949	23.013	0.0000	1.0000
BA – HtoM	10	−539.099 ± 0.100	1139.831	24.895	0.0000	1.0000
BA	11	−538.283 ± 0.089	1144.362	29.426	0.0000	1.0000
MP – HtoD	11	−540.129 ± 0.094	1148.054	33.118	0.0000	1.0000
ML	9	−550.259 ± 0.084	1155.988	41.052	0.0000	1.0000
BA – HtoD	10	−548.410 ± 0.108	1158.453	43.517	0.0000	1.0000

MarLik, marginal likelihood; k, number of parameters, that is, the number of transitions between sexual systems; BIC, Bayesian information criterion; PP, posterior probability for each model; Cum (PP), cumulative PP.
[a]Model codes indicate the reconstruction method and the transitions, which has been added/removed. BA, Bayesian mapping model; MP, maximum parsimony mapping model; ML, maximum likelihood mapping model; HtoM, transition from hermaphroditism to monoecy; GdtoD, transition from gynodioecy to dioecy; HtoD, transition from hermaphroditism to dioecy; GmtoD, transition from gynomonoecy to dioecy. A pictorial description of each model is showed in Figs 2 and S1.

be the result of lack of consideration by the model of processes at the inflorescence level, such as architectural constraints and resource competition between flowers and/or fruits (Torices & Méndez, 2010). The potential role of these inflorescence-level processes on sexual system evolution will be discussed in the next section.

This main pathway linking hermaphroditism, gynomonoecy and monoecy was not irreversible, as indicated by the presence of reversals. These reversals have also been inferred in tribal or subtribal studies within Asteraceae (e.g. Watson *et al.*, 2002 for the subtribe Artemisiinae; Torices & Anderberg, 2009 for the tribe Inuleae). Strikingly, all reversals showed a higher rate than that of their respective transition (Fig. 3f). This higher rate of reversals can be explained by taking into account the segregation of sexual systems among the two large groups of Asteraceae: the subfamily Asteroideae and the rest of the family or 'nonAsteroideae' grade (Fig. 4). In the 'nonAsteroideae' grade, gynomonoecy – and other sexual systems – have arisen in a hermaphroditic background. Reversals to hermaphroditism concentrate in the subfamily Asteroideae, where the gynomonoecious background is predominant (Fig. 4). Reversals from monoecy to gynomonoecy are more difficult to interpret, but probably indicate repeated transitions to gynomonoecy within a diverse monoecious clade, which arose from a single gynomonoecious ancestor. Further examination of these asymmetries in the evolution of sexual systems should consider the effect of sexual-system state on the diversification rate of these lineages. If some sexual systems would speed up the diversification rate of those lineages in which they are present, then transition

rates towards these sexual systems might be overrepresented because the proportion of taxa with a given state influences the inferred transition rates (Nosil & Mooers, 2005; Maddison, 2006; Goldberg & Igic, 2008). Unfortunately, there is insufficient information (phylogenetic and character state data) to apply models of character evolution that account for diversification effects for the whole family (see the Materials and Methods section).

A hypothesis for the evolution of nonhermaphroditic monomorphic sexual systems in Asteraceae

Selection for genetic recombination has been considered the main selective pressure for the evolution of sexual systems in flowering plants (Barrett, 2002; Charlesworth, 2006). In this line, the evolution of nonhermaphroditic sexual systems in Asteraceae has traditionally been interpreted as a mechanism promoting outcrossing (Burtt, 1977; Mani & Saravanan, 1999). Nevertheless, we believe that this cannot be the whole explanation, for two reasons. First, self-incompatibility is present in many nonhermaphroditic Asteraceae, which presumably eliminates the need for additional mechanisms to avoid selfing (Fryxell, 1957; Lloyd, 1979; Bertin & Kerwin, 1998; Bertin & Gwisc, 2002; Ferrer & Good-Avila, 2007). Secondly, any reasonable explanation of the evolution of nonhermaphroditic sexual systems in this family should explain the pervasive, striking sexual segregation within inflorescences, where female flowers are always placed in the outer positions and male flowers in the innermost positions (Fig. 5; Burtt, 1977; Mani & Saravanan, 1999). Sexual segregation within the capitula might reduce

(a) BA - GdtoD - 2 model. PP = 0.8734

Andromonoecy   Trimonoecy

0.39   0.33

Hermaphroditism — 0.93 / 2.74 — Gynomonoecy — 1.04 / 4.94 — Monoecy — 0.66 — Dioecy

0.24 — Gynodioecy — 6.23

(b) MP - GdtoD - 1 model. PP = 0.0529

Andromonoecy   Trimonoecy

0.39   0.40

Hermaphroditism — 0.68 / 3.23 — Gynomonoecy — 0.46 / 8.64 — Monoecy — 0.57 / 8.08 — Dioecy

0.21

0.23 — Gynodioecy — 1.38

(c) MP - HtoM & GmtoD model. PP = 0.0429

Andromonoecy   Trimonoecy

0.38   0.38

Hermaphroditism — 0.63 / 3.06 — Gynomonoecy — 0.68 / 8.85 — Monoecy — 0.83 / 8.93 — Dioecy

0.28

0.19 — Gynodioecy

(d) MP - GdtoD - 2 model. PP = 0.0186

Andromonoecy   Trimonoecy

0.42   0.41

Hermaphroditism — 0.90 / 3.63 — Gynomonoecy — 0.46 / 8.45 — Monoecy — 0.76 / 7.20 — Dioecy

0.25 — Gynodioecy — 1.44

(e) BA - GdtoD - 1 model. PP = 0.0104

Andromonoecy   Trimonoecy

0.38   0.33

Hermaphroditism — 0.92 / 2.75 — Gynomonoecy — 1.06 / 5.26 — Monoecy — 0.61 — Dioecy

0.06

0.22 — Gynodioecy — 7.24

(f) Model-averaged estimate of transition rates. PP = 0.9938

Andromonoecy   Trimonoecy

0.39   0.34

Hermaphroditism — 0.90 / 2.79 — Gynomonoecy — 0.98 / 5.36 — Monoecy — 0.66 / 0.94 — Dioecy

0.02

0.24 — Gynodioecy — 5.62

Fig. 3 Transition rates between sexual systems for the best five models of sexual system evolution in Asteraceae and the model-averaged estimate of transitions rates. (a) 'BA – GdtoD – 2 model'; (b) 'MP – GdtoD – 1 model'; (c) 'MP – HtoM & GmtoD model'; (d) 'MP – GdtoD – 2 model'; (e) 'BA – GdtoD – 1 model'; (f) 'Model-averaged estimate of transition rates'. Dashed arrows indicate reversals. In each model the posterior probability (PP) is shown. BA, Bayesian analyses; MP, maximum parsimony; GdtoD, transition from gynodioecy to dioecy; HtoM, transition from hermaphroditism to monoecy; GmtoD, gynomonoecy to dioecy.

geitonogamy and enhances outcrossing (Harder *et al.*, 2000), with outer seeds more outcrossed than inner ones. This was the case in some species (Marshall & Abbott, 1984; Cheptou *et al.*, 2001), but not in others (Gibson & Tomlinson, 2002; Gibson, 2001). Data are still too meagre to confirm the outcrossing hypothesis as an explanation of sexual segregation within capitula and the evolution of the monomorphic sexual system in this family. Further studies on differences in outcrossing rates at different flower positions within capitula are needed.

Instead, we suggest that evolution of nonhermaphroditic monomorphic sexual systems in Asteraceae might also be the result of sexual specialization, in line with the selection of packaging strategies proposed by Lloyd (1979). We hypothesize that floral sexual specialization is mediated by resource gradients within capitula that are a consequence of the centripetal anthesis of flowers (Burtt, 1977). Within capitula, as in other inflorescences, both resource competition among developing flowers and/or fruits (Stephenson, 1981) and architectural constraints (*sensu* Diggle, 2003) produce a decreasing resource gradient from the outermost (first) to the innermost (last) flowers (Torices & Méndez, 2010). Thus, outer seeds can reach larger sizes in the outer, compared with the inner part of the capitula (Rai & Tripathi, 1987; Kigel, 1992; Imbert *et al.*, 1997; Ruíz de Clavijo, 2000; El-Keblawy, 2003; Picó & Koubek, 2003; Torices & Méndez, 2010). Under this situation, sex allocation theory forecasts that those flowers in 'optimal positions' (Mazer & Dawson, 2001) will allocate proportionally more resources to female structures, whereas flowers in 'suboptimal positions' (*sensu* Primack & Lloyd, 1980) should become relatively male-biased. In fact, independently of the sexual system, femaleness decreases towards the capitulum centre (Fig. 5). We are currently testing the validity of our hypothesis in the tribe Inuleae and in a broad sample of Asteraceae. Other suggestions, such as sex specialization mediated by differential florivoy, also deserve attention (Bertin *et al.*, 2010).

Transitions to dioecy in Asteraceae

Dioecy has evolved independently many times among unrelated taxa in angiosperms. It also appears to have evolved along different pathways (Fig. 1a), although two of those

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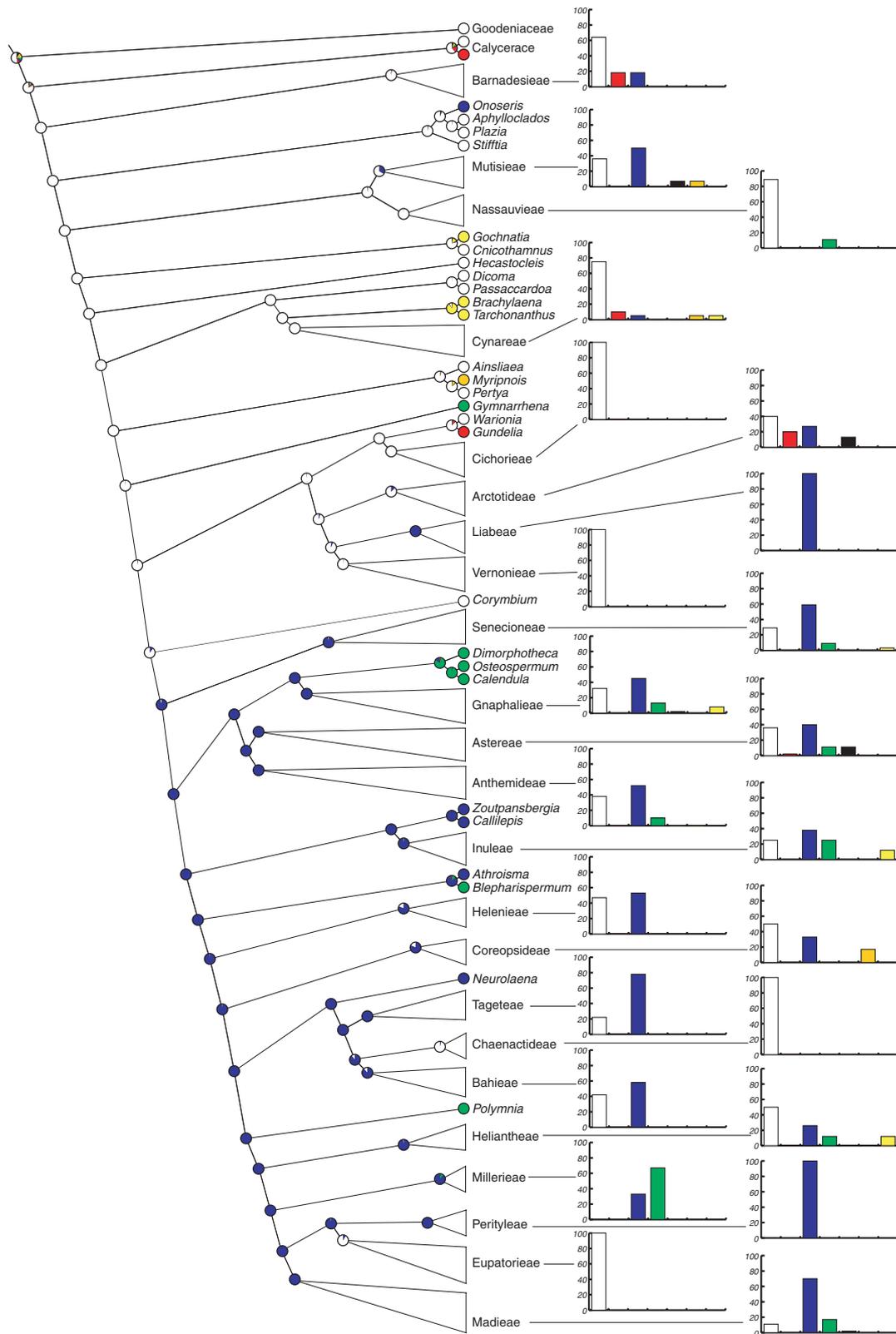
Fig. 4 Evolution of sexual systems within Asteraceae under maximum likelihood criterion. Probabilities at each node are reported as proportional likelihoods for each state (sectors of the pie charts) using the one-parameter model. White, hermaphroditism; red, andromonoecy; blue, gynomonoecy; green, monoecy; black, trimonoecy; orange, gynodioecy; yellow, dioecy. Sexual system frequency within collapsed clades is shown as bar charts.
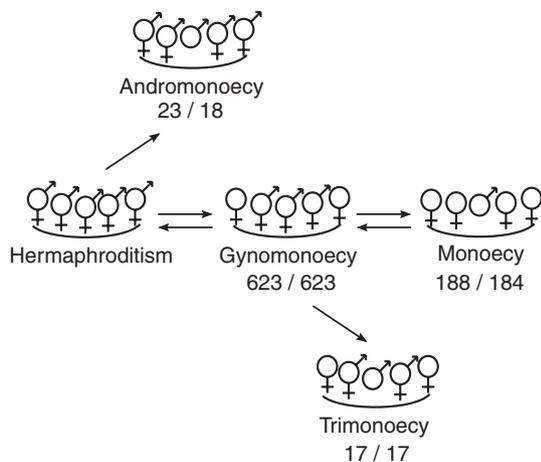
Fig. 5 The evolution of monomorphic sexual systems in Asteraceae from a hermaphroditic ancestor (Torices, 2009). The position of hermaphroditic, male and female flowers within the capitulum is shown. Numbers below each sexual system show the number of genera in which some species have that sexual system and the number of those genera in which the positional pattern in floral gender was fitted.

pathways are considered the main origins of dioecy in angiosperms: from monoecy and from gynodioecy (Bawa, 1980; Freeman *et al.*, 1997; Webb, 1999; Weiblen *et al.*, 2000; Barrett, 2002). Our phylogenetic model of evolutionary transitions between sexual systems in the evolution of Asteraceae supported these two main pathways to dioecy (Fig. 3f). Monoecy, gynodioecy and dioecy coexist in many other angiosperm families (M. Méndez, unpublished). However, to the best of our knowledge this is the first time both pathways to dioecy are phylogenetically inferred within the same lineage, which allows us to compare directly the importance of both pathways. The monoecious pathway accounted for a higher absolute number of changes (Fig. 2), but the transition rate from monoecy to dioecy was much lower than that from gynodioecy to dioecy (Fig. 3f). Thus, once evolved, transitions from gynodioecy to dioecy would be very fast (Fig. 3f), at least much more so than from monoecy to dioecy.

Regarding the monoecious pathway, our model supported a gradation between different sexual systems (Fig. 3f). Therefore, our results concerning the relationships between all sexual systems involved in this pathway partially matched the expectations derived from the sexual selection theory for the evolution of dioecy (Fig. 1b; Willson, 1979; Bawa & Beach, 1981), in that the inferred transitions between sexual systems followed a gradient in floral gender specialization (hermaphroditism–gynomonoecy–monoecy–dioecy). The gradation between hermaphroditism and dioecy was not unidirectional (Fig. 3f), and the reversals seem even more likely than direct transitions.

In addition, our averaged model included a direct origin of dioecy from hermaphroditism, but with a very low

change rate (Fig. 3f). A direct pathway from hermaphroditism to dioecy has been previously proposed by Ross (1982). The observed direct transitions from hermaphroditism to dioecy could be a spurious pattern resulting from a failure to include extant or extinct taxa with additional sexual systems brigding the gap between hermaphroditism and dioecy, or a loss of transitional intermediate stages within the ancestral species, if the character change rate exceeded the speciation rate, particularly along the gynodioecious pathway, which showed a very high transition rate to dioecy. We consider unlikely a spurious result based on omission of extant gynodioecious taxa because our sampling included a proportion of gynodioecious taxa that was larger than the proportion of gynodioecious genera in the family (Table 1). Instead, the high transition rate from gynodioecy to dioecy suggests a fast transition to dioecy through the gynodioecious pathway. Additionally, in the best model, this transition was totally missing (Fig. 3a).

In summary, our findings show that monoecy probably evolved from gynomonoecy in Asteraceae rather than from andromonoecy, and that dioecy evolved from both gynodioecy and monoecy. Furthermore, we found for the first time that monomorphic sexual systems were evolutionarily linked by means of a gradient of floral sex specialization. In addition, sexual segregation within capitula is pervasive in nonhermaphroditic sexual systems in Asteraceae and shows a consistent pattern of increased maleness towards the capitulum centre. We hypothesize that these position effects within inflorescences, mediated by resource competition among developing fruits and/or by architectural constraints, might have favoured the evolution of unisexual flowers, increasing the presence of nonhermaphroditic sexual systems in this successful lineage of flowering plants. This hypothesis could also be useful for other families where there is a concordance between positions patterns in floral gender and in fruit set.

Acknowledgements

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Pictorial description of models included in the model selection analysis.

Table S1 Number of estimated changes among sexual systems in Asteraceae with different priors and different branch lengths for the Bayesian analysis

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