

INVITED REVIEWS AND SYNTHESSES

On the rarity of dioecy in flowering plants

JOS KÄFER,* GABRIEL A. B. MARAIS* and JOHN R. PANNELL†

*Laboratoire de Biométrie et Biologie Evolutive, CNRS, UMR 5558, Université Lyon 1, Bât. Grégor Mendel 43, bd du 11 novembre 1918, 69622 Villeurbanne cedex, France, †Department of Ecology and Evolution, University of Lausanne, Biophore Building, 1015 Lausanne, Switzerland

Abstract

Dioecy, the coexistence of separate male and female individuals in a population, is a rare but phylogenetically widespread sexual system in flowering plants. While research has concentrated on why and how dioecy evolves from hermaphroditism, the question of why dioecy is rare, despite repeated transitions to it, has received much less attention. Previous phylogenetic and theoretical studies have suggested that dioecy might be an evolutionary dead end. However, recent research indicates that the phylogenetic support for this hypothesis is attributable to a methodological bias and that there is no evidence for reduced diversification in dioecious angiosperms. The relative rarity of dioecy thus remains a puzzle. Here, we review evidence for the hypothesis that dioecy might be rare not because it is an evolutionary dead end, but rather because it easily reverts to hermaphroditism. We review what is known about transitions between hermaphroditism and dioecy, and conclude that there is an important need to consider more widely the possibility of transitions away from dioecy, both from an empirical and a theoretical point of view, and by combining tools from molecular evolution and insights from ecology.

Keywords: angiosperms, Dollo's law, evolutionary dead end, leaky dioecy, reproductive assurance, sexual systems

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Introduction

Most flowering plant species (angiosperms) are hermaphroditic (Yampolsky & Yampolsky 1922; Barrett 2002; Charlesworth 2006) but deviations from this general pattern are frequent (see Fig. 1). Dioecy, that is the occurrence of separate male and female individuals, is found in about 6% of angiosperms (Renner & Ricklefs 1995; Weiblen *et al.* 2000; Renner 2014), and is thus strikingly rare in comparison with the equivalent sexual system (gonochorism) in animals, of which approximately 95% of all species have separate females and males (Jarne & Auld 2006; Eppley & Jesson 2008). However, while dioecy is rare in plants, it has nevertheless frequently evolved from hermaphroditism; in a recent assessment, Renner (2014) estimated that the 15 600 dioecious species may have evolved in between 900 and 5000 independent transitions from hermaphroditic

ancestors. Dioecious species thus typically form isolated lineages and rarely large species-rich clades (Darwin 1884; Yampolsky & Yampolsky 1922; Renner & Ricklefs 1995; Heilbuth 2000; Renner 2014), as becomes clear from its phylogenetic distribution: almost half of all families and the majority of orders contain dioecious species (Fig. 2), yet dioecy is almost always rare within these higher taxa.

The multiple origins of dioecy have attracted a great deal of attention from both empiricists and theoreticians, because they expose questions concerning the evolution and determination of separate sexes per se (Charnov *et al.* 1976; Bull 1983; Kohn 1988; Beukeboom & Perrin 2014), of contrasting allocation strategies and sex ratios (Hardy 2002; West 2009), as well as questions about sexual dimorphism and sexual selection in plants (Charlesworth 1999; Webb 1999; Barrett 2002; Moore & Pannell 2011; Barrett & Hough 2013). Some dioecious plants have also recently evolved sex chromosomes, either XY or ZW systems, which are found in many animals, too (Westergaard 1958; Ming *et al.* 2011; Bachtrog

Correspondence: Jos Käfer, Fax: (+33)(0)4 72 43 13 88; E-mail: jos.kafer@univ-lyon1.fr

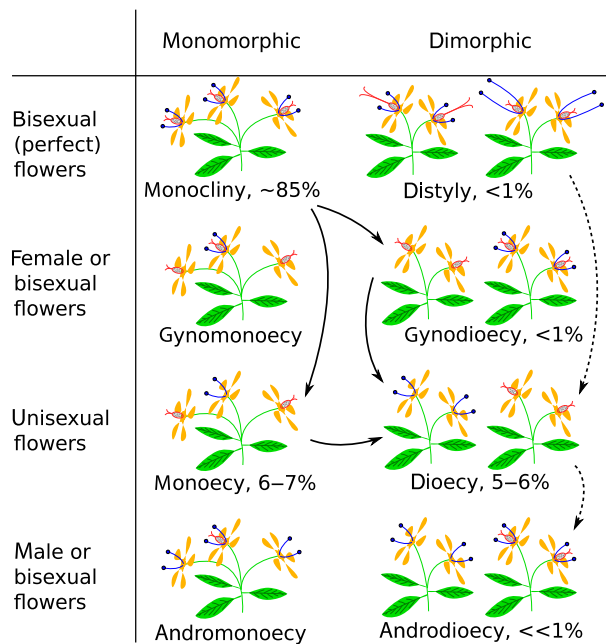


Fig. 1 Schematic depiction of the various conformations of combined vs. separate sexes in plant populations. Estimates for the percentage of angiosperm species corresponding to each conformation are given below each diagram, taken from Renner (2014). Note that the term “hermaphroditism” is ambiguous, because it can either refer to the flowers or the individuals. In the latter sense, all monomorphic species can be considered hermaphroditic. Conformations having more than two types of flowers or individuals exist, but are probably rare, and are not indicated here. The arrows correspond to the main pathways that have been identified (see Box 1): plain arrows indicate the gynodioecy and the monoecy–paradioecy pathways, that have received considerable attention, and dashed arrows the pathways for which more limited evidence exists.

et al. 2014), and with which they share some evolutionary processes (Charlesworth *et al.* 2005; Charlesworth 2016; Muyle *et al.* 2017). Ever since Darwin (1884) discussed dioecy from an evolutionary perspective, a number of hypotheses have been proposed and examined for why dioecy has evolved (cf., among many others, reviews by Bawa 1980; Thomson & Brunet 1990; Sakai & Weller 1999; Charlesworth 1999; Renner 2014). In contrast, questions arising from the relative rarity of dioecy and its scattered phylogenetic distribution have received much less attention.

Given the frequent evolution of dioecy and its overall rarity in angiosperms, the view that dioecious species are less evolutionary successful than hermaphrodites has dominated the literature. In his now classic review of plant sex chromosomes, Westergaard (1958) dismissed dioecy as a “failure” in plants. This view, which has only seldom been challenged (e.g. Bawa 1980; Renner 2014), was bolstered by a quantitative comparative

analysis conducted by Heilbut (2000), who also concluded that dioecy was an “evolutionary dead end”, perhaps easily acquired but doomed to failure as a result of higher lineage extinction rates, resulting in shorter persistence times of those lineages. Similar reasoning and analysis has explained the distribution of self-compatible plant species (Stebbins 1950; Iqic *et al.* 2008; Goldberg *et al.* 2010), or of asexual species (Maynard Smith 1978; Barraclough *et al.* 2003; Schwander & Crespi 2009): while self-incompatibility and sexuality are complex traits that are more easily lost than regained, they nevertheless are represented in the majority of extant species due to higher extinction rates of the species that lost them.

Recently, however, two studies have called into question the view that dioecy is an evolutionary dead end. First, building upon an improved test for sister clade comparisons (Käfer & Mousset 2014), Käfer *et al.* (2014) documented a small positive effect of dioecy on species richness, suggesting that diversification might be more rapid in at least some of the dioecious lineages than in the hermaphrodite lineages from which they are derived. Second, using recently developed phylogenetic tools (Maddison *et al.* 2007; FitzJohn *et al.* 2009), Sabath *et al.* (2015) found no general effect of dioecy on diversification rates in a number of genera having both dioecious and nondioecious species, concluding that different lineages reveal different patterns. If dioecy does evolve frequently, and has no consistently negative effect on diversification, why is it not more common than it is?

We here promote the view that the rarity of dioecy in flowering plants might be due not to its frequent loss by extinction, but because dioecious species sometimes, perhaps often, revert back to an hermaphroditic state. Although the evolution of dioecy has frequently been considered irreversible, partially based on comparisons with gonochorism in animals (Bull & Charnov 1985; but see Bachtrog *et al.* 2014), recent studies suggest that reversals might be common in plants (Barrett 2013; Käfer *et al.* 2014; Renner 2014). We review the sparse literature bearing on the question of reversion of dioecy to hermaphroditism in flowering plants and argue that this topic needs more attention. We begin with a short overview of ideas about the evolution of dioecy, the paths that the transition has taken, and the (selective) drivers that might be responsible. In this context, we argue that evolution has taken different routes towards dioecy, and that quite different evolutionary forces have probably been involved in different lineages. We should therefore be cautious about trying to generalize across all flowering plants. Next, we explain the basis of the dead-end hypothesis for dioecy, and argue that, at least for a majority of lineages, it provides a poor

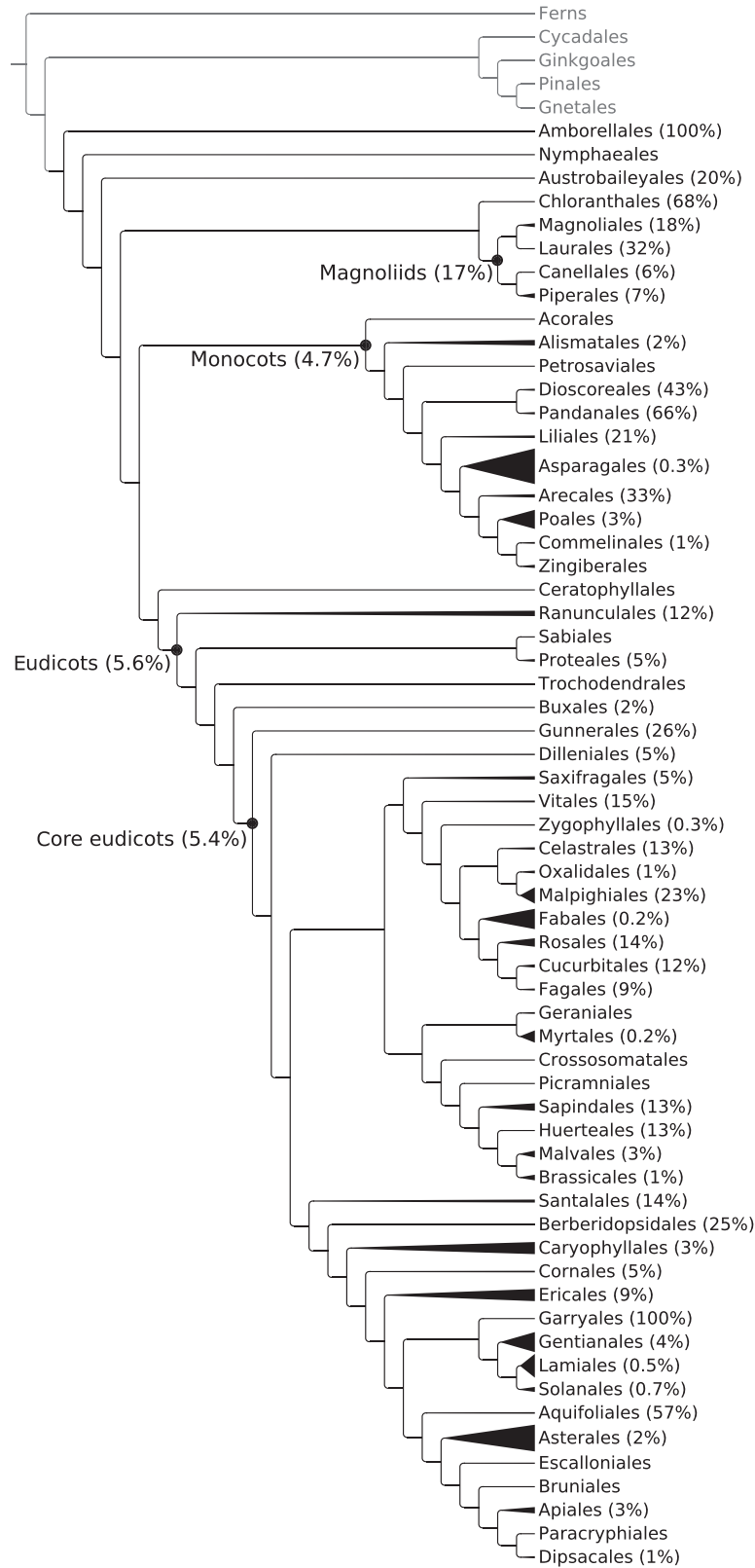


Fig. 2 Distribution of dioecious species among the angiosperm orders. The angiosperm tree is reproduced from Stevens (2001 onwards). Differences in clade size, varying from 1 (Amborellales) to 36 135 species (Asparagales), are represented by the size of the triangles. The percentage of dioecious species, if any, is indicated in parentheses.

explanation for the phylogenetic distribution of dioecy among flowering plants. We then examine the alternative hypothesis that dioecy is rare because of frequent reversions to hermaphroditism, first exploring the phylogenetic evidence for this idea and then considering what mechanisms might have been involved.

Evolution of dioecy in flowering plants: multiple paths and drivers

Not only is dioecy widely distributed among higher taxa of flowering plants (Fig. 2), it is also associated with a number of different traits or features (cf. Bawa 1980; Givnish 1980; Muenchow 1987; Thomson & Brunet 1990; Charlesworth 1993; Renner & Ricklefs 1995; Sakai & Weller 1999; Vamosi *et al.* 2003). For instance, in comparison to non-dioecious species, dioecious species are more likely to be trees, shrubs or lianas than annual herbs; they have higher frequencies in the tropics and on islands than in temperate regions or on continents; they are more often pollinated by wind or water than by insects and particularly rarely by bats or birds; they rely more often on animal-mediated seed dispersal than on dispersal by other means; they tend to have small, simple whitish or greenish flowers in large inflorescences rather than large flowers with complex morphology; and they tend to occur in clades where species with combined sexes are monoecious, that is, individuals have separate male and female flowers. The number of different traits, the fact that none of them is found in a large majority of dioecious species and the dependence of several traits upon one another (e.g. trees more often have animal-dispersed fruits and occur in the tropics) make the search for a general reason for the evolution of dioecy challenging.

Dioecy could have two main advantages over hermaphroditism for the organisms that possess it, and these advantages could explain at least some of the associations. First, dioecy could evolve as a mechanism to ensure outcrossing, which is thought to be beneficial for most species (Darwin 1876; Glémin 2007; Wright & Barrett 2010). That outcrossing is a major driving force for plant reproductive system evolution is also suggested by the fact that at least half of the flowering plant species have some mechanism to avoid selfing (Igic & Kohn 2006). Thus, dioecy, as a mechanism that prevents selfing, could replace genetic self-incompatibility (SI) under certain conditions (Lloyd 1974; Charlesworth & Charlesworth 1978). In the case of wind-pollinated plants, or in the case of large or clonal plants that present many open flowers at the same time, pollen may be less effectively transferred from one plant to the other, leading to higher selfing rates (Bawa 1980; Harder & Barrett 1995; Barrett 2015). Genetic self-incompatibility

in such plants would prevent self-fertilization, but would not prevent self-pollination and pollen discounting, that is, it may still compromise outcrossing opportunities through the loss of pollen to self-pollination (Holsinger 1988; Harder & Wilson 1998), as well as the clogging of stigmas. Also, as SI is probably much harder to evolve than to lose (Goldberg *et al.* 2010; Barrett 2013), the loss of SI might set the stage for the evolution of dioecy as an alternative outcrossing mechanism, as appears to have happened after the loss of SI due to polyploidization (Miller & Venable 2000; Ashman *et al.* 2013). Moreover, SI might be lost for the sake of reproductive assurance when species colonize isolated areas such as Oceanic islands, and subsequent selection against selfing might explain the high incidence of dioecy on many such islands (Baker 1955; Carlquist 1966; Pannell 2015).

Second, dioecy could be a way to optimize resource allocation for the female and the male functions, which is an important hypothesis for the evolution of gonochorism in animals (Maynard Smith 1978; Charnov 1982). Charnov *et al.* (1976) showed that hermaphroditism is favoured when the male or female fitness of individuals flattens with accrued investment; indeed, the immobility of plants is likely to limit male fitness more than in mobile animals (Eppley & Jesson 2008). Furthermore, the investment into attractive flowers may benefit both sexual functions, while the main costs of male and female functions are separated in time, favouring hermaphroditism (Charnov *et al.* 1976). Sex allocation theory could thus explain why wind-pollinated species, which invest less in showy flowers, are more often dioecious than insect-pollinated species (de Jong & Klinkhamer 2005), or why species with fleshy fruits, in which the costs of reproduction are unevenly distributed between the sexes, have a greater tendency to become dioecious (Bawa 1980; Charnov 1982). Whether dioecy should mainly evolve to avoid selfing or to optimize sex allocation has been the subject of heated debates in the past (Charlesworth & Charlesworth 1978; Willson 1979; Bawa 1980; Thomson & Barrett 1981; Charnov 1982; Givnish 1982; Charlesworth 1985), but recent surveys and modelling studies suggest that the evolution of dioecy probably results from an interplay of several forces that include inbreeding avoidance and advantages associated to sexual specialization (Dufay & Billard 2012; Spigler & Ashman 2012; Dornier & Dufay 2013; Golenberg & West 2013; Litrico & Maurice 2013; Sinclair *et al.* 2013).

The question of *why* dioecy evolves is intimately linked with the question of *how* it evolves. A simple and insightful view is that the evolution of separate sexes from ancestors with perfect flowers requires at least two steps (see Fig. 1, Box 1): either dioecy evolves

with the spread of individuals of one sex first, and then the other (gynodioecy and androdioecy pathways), or it evolves starting with a transition of perfect-flowered hermaphrodites to monoecy, where individuals have unisexual flowers of both sexes, and then by the evolution of unisexual individuals (monoecy–paradioecy pathway). As discussed in Box 1, modelling suggests that the gynodioecy pathway is particularly likely to occur when the selfing rate and the level of inbreeding depression are both high; androdioecy, by contrast, cannot easily evolve in response to selection for inbreeding avoidance, because selfing severely compromises male siring opportunities and thus selects against males. The monoecy–paradioecy pathway, for which fewer theoretical studies exist, might proceed in response to selection for sexual specialization (Charnov *et al.* 1976; Charlesworth & Charlesworth 1981; Charnov 1982).

Which of the pathways is more important to explain the current distribution of dioecious species remains an open question. The fact that males of dioecious species more often develop female flowers or organs than the inverse (i.e. males are typically more likely to be ‘inconstant’ in their sex expression) indicates that males often have evolved after females via a gynodioecious intermediate state (Lloyd & Bawa 1984; Barrett 1992a; Ehlers & Bataillon 2007). Furthermore, detailed studies of gynodioecious species have revealed that there is substantial variation in the maleness of hermaphrodites, suggesting that different species may be at different intermediate stages along this pathway (Delph 1990; Barrett 1992a; Delph & Wolf 2005; McCauley & Bailey 2009). However, while the statistical association between dioecy and gynodioecy at higher taxonomical levels has been shown recently (Dufay *et al.* 2014), the support for the association between dioecy and monoecy is stronger (Renner & Ricklefs 1995). Furthermore, many gynodioecious species are probably not en route to becoming fully dioecious (Darwin 1884; Renner 2016; Rivkin *et al.* 2016), and gynodioecy is associated with quite different traits than is dioecy, as gynodioecy is for example more frequent in herbaceous plants and temperate climates (Dufay *et al.* 2014; Caruso *et al.* 2016; Rivkin *et al.* 2016), whereas dioecy is more common in woody species in the tropics (Renner & Ricklefs 1995). Monoecy, on the other hand, is associated with some of the same traits as dioecy, such as wind pollination (Charlesworth 1993; Renner & Ricklefs 1995; Linder 1998; Friedman & Barrett 2008), suggesting that dioecy has more often evolved under conditions that favour monoecy than those that favour gynodioecy. It should finally be noted that the evolution of dioecy from hermaphroditism does not necessarily follow two distinct steps; evolution can be gradual as well or require more steps (Golenberg & West 2013; Renner 2016). For example, dioecy in the aquatic

perennial *Sagittaria latifolia* seems to have evolved from monoecy via gynodioecy (Dorken & Barrett 2004), where hermaphroditic individuals in such gynodioecious populations do not have perfect flowers, but separate male and female flowers (cf. Renner & Won 2001). In contrast to this hypothesis, recent evidence suggests that gynodioecy in this species may also be the result of hybridization between monoecious and dioecious populations (Yakimowski & Barrett 2016) and might thus not have been a direct step towards dioecy from monoecy.

End of the dead-end hypothesis?

The dead-end hypothesis for the evolution of dioecy posits that while dioecy may evolve frequently, under a wide range of circumstances, dioecious lineages do not persist and become evolutionary sinks or dead ends, much as is thought to be the case for species that lose self-incompatibility (Stebbins 1950; Escobar *et al.* 2010; Goldberg *et al.* 2010) or those that evolve asexual reproduction (Maynard Smith 1978; Paland & Lynch 2006; Schwander & Crespi 2009). Support for the dead-end hypothesis was bolstered by Heilbut (2000), who provided quantitative evidence showing that dioecious clades contained fewer species than their nondioecious sister clades, and thus apparently diversify less well (see Box 2).

Subsequent work suggested that the reduced diversification rate of dioecious lineages was mainly due to higher extinction rates of dioecious species. Heilbut *et al.* (2001) proposed a demographic model that might explain the poor success of dioecious lineages. If seeds are dispersed over short distances into small seed shadows where establishing individuals experience strong competition, dioecious species would suffer a ‘seed-shadow handicap’, because only females disperse seeds and the potential seed shadow of males remains unexploited (Heilbut *et al.* 2001). Dioecious species may consequently be more prone to extinction via demographic stochasticity. This demographic disadvantage could be overcome if females produce sufficiently more and/or better-dispersed seeds than hermaphrodite competitors, a prediction that might account for the frequent occurrence of fleshy fruits in dioecious species (Vamosi *et al.* 2007).

In a second model, Vamosi & Otto (2002) considered the demographic consequences of the possible effect of dioecy on interactions with pollinators. Competition among males for pollinators could render males more attractive than females (Bateman’s principle; Bateman 1948; Moore & Pannell 2011), to the extent that visits to females may become insufficient to guarantee seed set, which increases the population extinction risk (Vamosi & Otto 2002). Indeed, experimental studies suggest that

Box 1. Pathways to dioecy

It has been long acknowledged that many dioecious species have recent hermaphroditic ancestors (Darwin 1884), because they are closely related to hermaphroditic taxa (see Introduction), and because many unisexual flowers still show residues of the aborted organs of the other sex (Mitchell & Diggle 2005). Early on, it was suggested that other sexual systems (see Fig. 1) might sometimes be intermediate steps in the evolution of dioecy from hermaphroditism (Yampolsky & Yampolsky 1922). Influential studies in the second half of the 20th century identified gynodioecy and monoecy as the most probable candidates for these intermediate stages (Charlesworth & Charlesworth 1978; Renner & Ricklefs 1995).

Gynodioecy pathway

According to the model by Charlesworth & Charlesworth (1978), a nuclear male-sterility mutation can spread if the product of the inbreeding depression and the selfing rate exceeds 0.5. Thus, the main driving force for the first step of this pathway is the existence of inbreeding depression in a population of partially selfing hermaphrodites (Lewis 1941; Lloyd 1974; Charlesworth & Charlesworth 1978). However, the complete loss of the male reproductive function severely decreases the individual's contribution to the next generation, and the mere gain of seed quality through outcrossing may often be not enough to compensate this reduction. Females may also be able to invest more resources to produce more and/or better seeds, which could probably easily be achieved because they do not produce pollen.

Females need a much smaller advantage to invade hermaphroditic populations in the case of cytoplasmic male sterility (CMS), because feminizing cytoplasmic selfish elements are transmitted to all offspring of a female (Lewis 1941; Lloyd 1974). The frequency of females in such gynodioecious populations can be high, which could facilitate the next step towards dioecy, that is either the invasion of males or selection to increase the maleness of the hermaphrodites (Maurice *et al.* 1994; Schultz 1994). There is indeed evidence that many cases of gynodioecy are caused by CMS (Bailey & Delph 2007; Delph *et al.* 2007), but there is little direct evidence that CMS-mediated gynodioecy has preceded the evolution of dioecy (Spigler & Ashman 2012).

Androdioecy

The spread of a female-sterility mutation in a hermaphroditic population, and thus the establishment of an androdioecious population, occurs under much more stringent conditions than the spread of male sterility, as pure males would have to compete with selfing hermaphrodites to sire ovules (Lloyd 1975c; Charlesworth & Charlesworth 1978). Also, male sterility immediately prevents self-fertilization of the ovules of an individual, while female sterility does not: gynodioecy thus more effectively promotes outcrossing than androdioecy (Lloyd 1976). The relative frequencies of gynodioecy and androdioecy are consistent with the theory: the former is much more common than the latter, which is extremely rare and found only in a handful of species, mostly resulting from the breakdown of dioecy (Charlesworth 1984; Pannell 2002). Nevertheless, there is now convincing evidence that androdioecy can evolve from hermaphroditism through the spread of female-sterility elements due to previously unanticipated mating patterns (Pannell & Korbecka 2010; Saumitou-Laprade *et al.* 2010; Billiard *et al.* 2015), yet this seems to be rather an exception than a rule.

Monoecy–paradioecy pathway

In this pathway, individuals first evolve separate male (staminate) and female (pistillate) flowers, and selection subsequently favours a gradual tendency of some individuals to accentuate their male and others their female function (Lloyd 1972a, 1980; Renner & Won 2001). There has been much less interest in this pathway by theoreticians than in the gynodioecy pathway. It is nevertheless thought that the evolution of dioecy via monoecy might proceed in response to selection for increased sexual specialization (Charnov *et al.* 1976; Charlesworth & Charlesworth 1981; Charnov 1982), and not in response to selection for inbreeding avoidance, which should favour a gynodioecious step, as noted above. The separation of the sexes in this pathway is more gradual than in the gynodioecy pathway: female flowers are converted into male flowers on some individuals, and male flowers into female flowers on others, such that there is no sudden reduction in reproductive output due to sterility mutations (Lloyd 1972a, 1980). This putative path has been put forward as an explanation for the statistical association at the

Box 1. Continued

level of the angiosperms between monoecy and dioecy (Renner & Ricklefs 1995), which is indeed stronger than the one between gynodioecy and dioecy (Dufay *et al.* 2014).

Other pathways

Although gynodioecy and monoecy are seen as the two most likely alternative intermediate stages in the evolution of dioecy from hermaphroditism, dioecy may also evolve from distyly or heterodichogamy, in which populations are initially polymorphic for the spatial or temporal separation of the sexes on plants, respectively (Darwin 1884; Lloyd 1979; Ross 1982; Webb 1999; Pannell & Verdu 2006). In distylous species, hermaphroditic flowers either have long styles and short stamens, or long stamens and short styles, with mating usually restricted to crosses between rather than within each of the morphs by a linked self-incompatibility system (Baker 1958; Ornduff 1966; Barrett 1992b). Here, dioecy may evolve if one of the morphs (usually that with long styles) evolves towards increasing female function and the other (usually the short-styled morph) becomes increasingly male. In heterodichogamous species, populations comprise protandrous and protogynous individuals (usually at equal frequencies), where half the individuals flower first as a male and then as a female, and vice versa (Renner 2001). Disruptive selection can give rise to the evolution of dioecy via a gradual shift towards increased maleness (e.g. in protandrous individuals) or femaleness (in protogynous individuals; Pannell & Verdu 2006).

male flowers are often preferred by pollinators (e.g. Bell 1985; Waelti *et al.* 2009), and comparative evidence indicates that insect-pollinated plants are under-represented among dioecious species (see above). Together, these models thus not only suggest that dioecious species might be more likely to go extinct than their hermaphroditic counterparts, but they also hint at how they could escape that fate through the evolution of life history or dispersal traits that reduce their handicaps. Indeed, Vamosi & Vamosi (2004) found a tendency for dioecious clades to have more species if they were tropical, or if their species had fleshy fruits, a woody growth form and less ornate flowers.

Although the dead-end hypothesis has frequently been cited to explain patterns of species richness (e.g. Magallon & Castillo 2009; Schlessman *et al.* 2014), as examples of species selection (e.g. Savolainen *et al.* 2002; Rabosky 2009), or in reviews on plant breeding systems (Barrett 2002; Charlesworth 2006), recent studies suggest that the hypothesis may have more limited support than is widely thought. First, Käfer & Mousset (2014) showed that sister clade comparisons can lead to incorrect conclusions if the trait under study is evolutionarily derived, as is likely to be the case for dioecy, because in most cases, it has probably evolved fairly recently from hermaphroditism. Using a new test for sister clade pairs with a known ancestral state (see Box 2), and trimming the data of Renner & Ricklefs (1995) so that it included only clade pairs for which dioecy was probably not the ancestral state, Käfer *et al.* (2014) found no evidence for the idea that transitions to dioecy reduced the diversification rate. Rather, their analysis pointed to the possibility that transitions to dioecy tend to lead to an increase in diversification, instead of a decrease.

The absence of a consistently negative effect of separate sexes on diversification was confirmed by Sabath *et al.* (2015), who fitted an explicit model of speciation, extinction and character transitions (Maddison *et al.* 2007; Fitz-John *et al.* 2009; see Box 2) to each of 43 dioecious genera. They too found a slight excess in genera for which dioecy seemed to be associated with a higher diversification rate, although the excess was not significant in their analysis. Both the studies by Käfer *et al.* (2014) and Sabath *et al.* (2015) found that there is large variation in the diversification rates of dioecious clades, which could be due to one or several other factors that were not taken into account in the analyses. However, the most common traits associated with dioecy (biotic dispersal, abiotic pollination, tropical distribution and woody growth form) were included in these studies, yet they could not explain the observed differences in diversification rates either. Thus, while the dead-end hypothesis has given rise to some interesting secondary hypotheses explaining why dioecious species might be more prone to extinction under some conditions (Heilbutth *et al.* 2001; Vamosi & Otto 2002; Vamosi *et al.* 2007), it does not seem to provide a general explanation for the rarity of dioecy and its scattered phylogenetic distribution.

Frequent reversion of dioecy to hermaphroditism?

The dead-end hypothesis predicts that dioecious lineages are more likely to go extinct than their hermaphroditic counterparts, but it also supposes that evolutionary paths to dioecy are one-way streets, that is that reversions are unlikely (although this assumption is not always made explicitly). This idea conforms to

Box 2. The study of diversification of dioecious clades

The analysis of Heilbuth (2000) that led to the dead-end hypothesis for dioecy was based on sister clade comparisons (cf. Mitter *et al.* 1988; Vamosi & Vamosi 2005). These consist of pairwise comparisons of the number of species between clades having the trait of interest and their most closely related clades that lack this trait. The two clades in each pair have the same age, which allows one to use species numbers alone to estimate differences in diversification, without having to worry about variation in diversification rates through time (Ricklefs 2007). Second, the correction for phylogenetic inertia is automatic, as one can reasonably suppose that both clades share a majority of traits with their common ancestor. The use of a large number of clade pairs and of statistical testing removes the effect of incidental associations between traits.

Despite substantial methodological improvements (cf. Vamosi & Vamosi 2005), sister clade comparisons did not explicitly consider the timing of the evolution of the trait under study; they thus implicitly assumed that the appearance of the trait coincided with the split of both clades from their common ancestor. Heilbuth's (2000) analysis of dioecy through sister clade comparisons also made this implicit assumption. However, we know that dioecy has mostly evolved recently from a hermaphrodite ancestor; it is thus likely to have evolved somewhere along the branch leading to the dioecious clade. This puts an extra constraint on the dioecious clade, namely that it should not have diversified before dioecy evolved. The assumption that both clades diversified for equal times, as mentioned above, is thus likely often violated.

Käfer & Mousset (2014) formalized the point that dioecy might evolve subsequent to a lineage split. They found that, in the case of derived traits like dioecy, the null expectation under equal diversification rates is that the clades with the derived trait should be smaller than their sisters. The new test they proposed corrects for the sampling bias that is introduced by "cherry-picking" clade pairs, but requires that ancestral states for all clade pairs are identified. Käfer *et al.* (2014) thus only used a set of 115 clade pairs for which the ancestral state was inferred to be non-dioecious. With this data set, including more than 50% of all known dioecious species, a small positive effect of dioecy on diversification was detected.

Sister clade comparisons use only a minimal amount of information that is present in phylogenies. More sophisticated methods that are explicitly based on molecular phylogenies have been developed in recent years. One particularly popular method, "BiSSE" (Binary State Speciation and Extinction model; Maddison *et al.* 2007; FitzJohn *et al.* 2009), allows the simultaneous estimation of speciation, extinction and transition rates. This method has, for example, been successfully applied to show that the loss of self-incompatibility in Solanaceae decreases the lineage diversification rate (Goldberg *et al.* 2010), and thus that self-compatibility is an evolutionary dead end. The rarity of dioecy in angiosperms, however, makes dioecy less suitable for the application of the BiSSE method. First, the power of BiSSE drops substantially when a trait is represented by less than 10% of the species in the data set (Davis *et al.* 2013). Furthermore, this rarity forces one to use large phylogenetic trees in order to have enough taxa for testing. However, the larger the phylogenetic trees, the higher the probability that other factors influence the diversification rates in the tree. These heterogeneities could lead to false inferences if they are not explicitly taken into account (Rabosky & Goldberg 2015).

Sabath *et al.* (2015) addressed these difficulties by focussing on a set of genera in which both dioecy and other sexual systems occur. They fitted a BiSSE model to each genus separately, minimizing the problems due to rate heterogeneity, and they corrected for the effect of residual heterogeneities through simulations. Furthermore, the average proportion of dioecious species in these genera is substantially higher than the angiosperm average, which provides a solution to the loss of power at low frequencies of the state. Sabath *et al.* (2015) did not find a significant signal for the hypothesis that dioecy would increase diversification rates, in contrast with the results of Käfer *et al.* (2014), but both studies refute the dead-end hypothesis as a general explanation for the rarity of dioecy.

Dollo's law, which states that evolution never reverts to exactly the same state as the ancestor (Gould 1970; Bull & Charnov 1985; Collin & Miglietta 2008). To some extent, the idea makes intuitive sense: the evolution of dioecy typically involves the spread of male- and female-sterility mutations (see Box 1), and the loss of a function should be more likely than regaining it. However, although sterility

mutations need to increase in frequency in a population evolving towards dioecy, they cannot of course go to fixation, as both sexual functions must continue to be expressed for sexual reproduction to take place. The recovery of the lost sexual function by members of one sex thus need not be so difficult if it simply requires the expression of genes that have continued to be expressed in

the genome of individuals of the other sex. Reversions from dioecy back towards hermaphroditism should therefore be developmentally and evolutionarily possible.

Although the topic has received too little attention, evidence for the breakdown of dioecy through the re-acquisition of the lost sexual function by each respective sex is accumulating. Recent research has established that dioecy most probably was the ancestral state of several large families, or even orders, in which hermaphroditism has re-evolved. For instance, Datwyler & Weiblen (2004) found dioecy to be ancestral in the Moraceae and the Urticaceae (Rosales); Zhang *et al.* (2006) assigned dioecy to the ancestor of the Begoniaceae, Cucurbitaceae, Datisceae and Tetramelaceae (Cucurbitales); Schlessman (2010) inferred that dioecy was the ancestral state in the Apiales; and Käfer *et al.* (2014) concluded that the ancestor of the Euphorbiaceae, Rafflesiaceae and Peraceae (Malpighiales) was likely dioecious. The fact that these clades are species-rich, and that dioecy only occurs in a minority of species in them, argues against the idea that the evolution of dioecy is irreversible, or an evolutionary dead end. In the well-studied family Cucurbitaceae, there is strong phylogenetic evidence that dioecy has been lost several times (e.g. Volz & Renner 2008; Schaefer & Renner 2010). It would thus seem that clades that start out as dioecious can, and sometimes do, end up being predominantly hermaphroditic following one or more reversions.

Of course, the loss of dioecy does not necessarily imply a return to the ancestral hermaphroditic state in which individuals have 'perfect' flowers, but may lead to the evolution of monoecy, in which individuals have separate male and female flowers. Of the clades just cited, only in the Apiales does dioecy seem to have reverted to hermaphroditism with perfect flowers (Schlessman 2010); the other clades are characterized by a high percentage of monoecious species. That monoecy often results from the loss of dioecy rather than being a precursor to dioecy was also inferred by Weiblen *et al.* (2000) in their analysis of sexual-system evolution in the monocots (comprising 11 orders, and about 60 000 species, cf. Fig. 2). They found that most transitions to dioecy were from hermaphroditic ancestors (26–35 transitions), while only a few (3–4) were from monoecious ancestors. On the other hand, monoecy was about equally likely to evolve from hermaphroditism (9–17 transitions) as from dioecy (9–11 transitions); transitions from dioecy to hermaphroditism were slightly rarer (6). Thus, the strong statistical association between dioecy and monoecy found by Renner & Ricklefs (1995) might often be due to evolutionary transitions from dioecy to monoecy, rather than the reverse.

It is not clear whether the preferential breakdown of dioecy to monoecy rather than to hermaphroditism may be attributable to developmental constraints. For

example, in the Cucurbitaceae, perfect flowers are very rare but not absent altogether (Schaefer & Renner 2011), and double loss-of-function mutants yield perfect flowers in the mostly monoecious melon (Boualem *et al.* 2015). The rarity of perfect flowers in this clade thus seems to reflect a lack of selection rather than a developmental constraint. In the Euphorbiaceae, inflorescences of unisexual flowers have formed so-called pseudanthia, clusters of highly reduced female and male flowers that, together, function as a floral unit similar to an hermaphrodite flower (cf. Prenner & Rudall 2007). This suggests that potential developmental constraints in this family may have prevented the reappearance of perfect flowers, but that these constraints also can be bypassed if selection favours hermaphroditic structures. Yet even in the Euphorbiaceae, perfect-flowered variants have sometimes been observed (Durand 1963), suggesting that the constraint cannot be absolute. It may thus be that monoecy is favoured over hermaphroditism after a history of dioecy in a given lineage because of evolved associations between unisexuality and other traits.

We currently lack a global picture and quantitative data on reversions from dioecy across the phylogeny of flowering plants. Phylogenetic studies are needed to address this lack of knowledge, but these will only yield indirect evidence of reversals, and macroevolutionary models of sexual-system evolution often yield ambiguous results that are difficult to interpret (e.g. Renner & Won 2001; Volz & Renner 2008; Torices *et al.* 2011). The fact that a large body of work exists on certain pathways (i.e. the monoecy and gynodioecy pathways towards dioecy) might influence what model is finally retained as more plausible and might obscure less well-known pathways. We thus expect that improved phylogenies and explicit acceptance of the idea that the evolution of dioecy is reversible may reveal many new reversals that could be investigated in greater detail. Of course, in some clades (e.g., the families Ebenaceae, Myristicaceae, Pandanaceae and Menispermaceae; Renner & Ricklefs 1995; Heilbuth 2000; Käfer *et al.* 2014; Renner 2014), dioecy does not appear to revert to hermaphroditism or monoecy, or does so only rarely; such clades are characterized by a large number of dioecious species, but few of them have been well studied (Renner 2014). It is thus likely that, in some lineages, a transition to dioecy does indeed reflect a one-way street, but it is not known why lineages should differ in this sense, too.

By what mechanisms could dioecy be lost?

Although we have a poor understanding from a phylogenetic point of view as to why some lineages do seem

to lock into a dioecious state whereas others allow reversion, there are three mechanistic hypotheses that might explain reversions when they do occur. The first two hypotheses are directly inspired by the selective forces that have been identified to play a role in the evolution of dioecy from hermaphroditism (discussed above), and a third may be specific only to the loss of separate sexes.

First, just as the shape of fitness gain curves in sex allocation theory can explain why dioecy evolves in some cases (Charnov 1982; West 2009), so fitness gain curves could also account for the evolution of hermaphroditism from dioecy. Models indicate that hermaphrodite phenotypes could invade a dioecious population under conditions where the male and/or female gain curves become saturating, or flatten off with investment (Charnov *et al.* 1976). There are reasons to suspect that male and female fitness gain curves must often be saturating in plants, notably if pollen grains from the same parent compete with one another to fertilize a restricted pool of ovules (Hamilton 1967; Taylor & Bulmer 1980; Charnov 1982; Lloyd 1983, 1984), or if seeds are dispersed into a restricted seed shadow, leading to competition among related seedlings (Clark 1978; Lloyd 1984). The loss of, or a change in, effective pollinators or seed dispersers, or a change in the density of individuals in plant populations could therefore favour hermaphroditic over male or female strategies. Unfortunately, data bearing on these ideas lag far behind the theory, and we still have a poor idea of the actual shape of male or female fitness gain curves. This is an area where the tools of molecular ecology could be profitably employed to infer parental fitness gains from the detailed genetic structure and genealogy of populations.

Second, in lineages in which dioecy is maintained as an outcrossing mechanism, it is conceivable that self-fertile hermaphrodites could invade a population if circumstances arose that caused a reduction in inbreeding depression. Genetic bottlenecks can purge a population of its genetic load (Glémin 2003), for example through recurrent colonization in a range expansion (Pujol *et al.* 2009; Peischl *et al.* 2013). The purging of genetic load and its (re-) accumulation can take place rapidly (Glémin 2003; Pujol *et al.* 2009), so one would need to compare patterns of inbreeding and inbreeding depression among dioecious and nondioecious populations of species displaying within-species variation in their sexual systems. Alternatively, when other means of outcrossing are acquired, the maintenance of the inbreeding depression need not to be sufficient to prevent the breakdown of dioecy. Such a possibility is suggested by studies on the Hawaiian genus *Schiedea* (Caryophyllaceae), in which sexual dimorphism

(gynodioecy and dioecy) is associated with a transition to wind pollination, possibly as a mechanism to avoid inbreeding (Weller *et al.* 1998). The occurrence of a hermaphroditic insect-pollinated species, *Schiedea lydgatei*, within the dimorphic, wind-pollinated clade, indicates that the transition might be easily reversible here. Although *S. lydgatei* suffers strong inbreeding depression, as its gynodioecious sister species, the fact that it relies on biotic pollination seems to increase its outcrossing rate, such that the product of selfing and inbreeding depression is not enough to maintain females in the population (Norman *et al.* 1997).

Finally, the evolution of self-fertile hermaphroditism from dioecy might be favoured under selection for reproductive assurance under conditions of mate and/or pollinator limitation (Maurice & Fleming 1995; Wolf & Takebayashi 2004; Pannell 2008). Dioecy represents an obligate outcrossing mechanism in which the absence of potential mates of the opposite sex should render an individual effectively sterile. If such situations should arise frequently, for example in species with a colonizing habit or where population densities diminish (Baker 1955; Pannell 2015; Pannell *et al.* 2015), males or females that produce a few hermaphroditic flowers, or flowers of the opposite gender, would have a clear advantage over those that are fully male or female (Pannell 1997a, 2000). The selection of hermaphroditism on the basis of the selfing ability of such 'leaky' or 'inconstant' males or females under conditions of mate limitation seems to represent the most likely scenario for the breakdown of dioecy and is starting to be supported by models and observations (Ehlers & Bataillon 2007; Crossman & Charlesworth 2014).

The classical examples for the breakdown of dioecy via inconstancy in sex expression are provided by the dioecious and monoecious species and populations in the genus *Leptinella* (Asteraceae; formerly named *Cotula*) studied by Lloyd (1972a,b, 1975a,b). Sex inconstancy is typically low in the dioecious populations, and plants with a few florets of the other sex are still functionally unisexual (Lloyd 1975a). However, in small populations where one of the sexes is lost, these inconstant individuals can have high relative fitness through the inconstant sex. If females are the heterogametic sex, as they are in these *Leptinella* populations (Lloyd 1975a), the selfing by inconstant females after a loss of males would result in the segregation of males in their progeny and thus the immediate restoration of dioecy. However, if the females are lost from such populations, selfing by males will yield only male progeny, and monoecy can evolve gradually as a result of selection on males to increase the production of female flowers. Due to secondary differences between male and female florets, Lloyd (1975b) was able to infer that

monomorphic populations with high levels of inconsistency were indeed mostly derived from inconstant males after the loss of females. Lloyd's experiments and observations suggest that loss of dioecy has occurred several times independently in different species of *Leptinella*.

Observations on the *Mercurialis annua* species complex (Euphorbiaceae) in Europe and the Mediterranean Basin also suggest that selection for monomorphism or dimorphism may depend on population densities and dynamics. Most *Mercurialis* species are dioecious perennials, and monoecy in the annual clade, including polyploid *M. annua*, is derived from ancestral dioecy (Durand & Durand 1992; Krähenbühl *et al.* 2002; Pannell *et al.* 2008). Where populations are small and population turnover (extinctions and colonization) is particularly frequent, monoecious individuals have a strong advantage over males and/or females through their ability to self-fertilize (Baker 1955; Pannell & Barrett 1998; Dorken & Pannell 2007; Pannell 2015). The mating system in monoecious *M. annua* is strongly density-dependent (Eppley & Pannell 2007), with isolated plants setting full seed set upon selfing but individuals in dense populations largely outcrossing (Hesse & Pannell 2011; Labouche *et al.* 2017). This allows males to co-occur with monoecious plants in androdioecious populations when populations grow in size, illustrating not only the selection of combined sexes when densities are low, but also the evolution of separate sexes when densities increase (Pannell 1997a; Pannell *et al.* 2014). In both dioecious and androdioecious *M. annua*, males are XY and females or monoecious individuals are XX (Russell & Pannell 2015), so that monoecious individuals can be interpreted as being modified females in which their 'leaky' sex expression (also observed in dioecious populations Yampolsky 1930; Kuhn 1939) has presumably been enhanced and canalized by selection. A scenario has been proposed where dioecious populations were located in southern refugia during the last ice age; monoecious individuals then colonized northwards when the climate got cooler, and finally, males are now spreading north among the dense monoecious populations (Pannell *et al.* 2014).

These examples show that whether and how selection can act to change the sexual system depends on the availability of inconstant sex expression in males or females, as well as on the mechanisms of sex determination. The breakdown of dioecy in *Leptinella* probably occurred through selection for enhanced leaky sex expression in homogametic males (Lloyd 1975b) and for selection of male flowers on homogametic females in *M. annua* (Pannell 1997b). While inconstant sex expression might be more frequent in species that have recently evolved dioecy, it also occurs naturally in species in which dioecy is old enough to have led to relatively strong sexual dimorphism and heteromorphic sex

chromosomes, such as in *Silene latifolia* (Frick & Cavers 1989) and *Humulus* (Ainsworth 2000). Mutations of the Y chromosome give rise to fertile hermaphrodites in *Carica papaya* (Ming *et al.* 2011), a species in a predominantly dioecious family (Carvalho & Renner 2012), as well as in *Silene latifolia* (Westergaard 1958; Lardon *et al.* 1999; Fujita *et al.* 2012). In these species, however, the Y chromosome is too degenerated to render the YY genotype viable, and models have shown that returns to monomorphism are not likely in this case (Ehlers & Bataillon 2007; Crossman & Charlesworth 2014).

Conclusion and future research directions

The widespread but scattered distribution of dioecy among flowering plants has puzzled evolutionary biologists: while the evolution of separate sexes has clearly been favoured in a wide range of circumstances, rarely has it led to large dioecious clades in plants, in sharp contrast to animals. While there does not seem to be a clear evolutionary advantage to dioecy for flowering plants, and while dioecy might even be a disadvantage for short-lived, insect-pollinated species with poor seed dispersal (Vamosi *et al.* 2003; Vamosi & Vamosi 2004), the idea that dioecy is typically an evolutionary dead end has recently been found wanting (Käfer *et al.* 2014; Sabath *et al.* 2015). We suggest that the distribution of dioecy in flowering plants, that is both its rarity and its occurrence in many families and orders, could be due not only to frequent gains, but also to frequent losses due to reversion rather than to extinction. While much effort has been invested in studying why and how dioecy should evolve, the question of whether, how and why it might be lost requires more attention.

More phylogenetic studies are needed to estimate how often reversions from dioecy to hermaphroditism have occurred, in which groups they are most frequent, and what ecological or life history traits might influence these reversals. The few case studies and models that are currently available point to the importance of leakiness, its heritability and the mechanisms of sex determination in dioecious species. Both field observations and experimental evolution studies could help to determine the frequency of leakiness in sex expression in the individuals of dioecious species, and the extent to which selection can act on this leakiness to bring about a change in the sexual system. It will be particularly important to know how inconstant sex expression affects the female and, especially, the male components of reproductive success and indeed the extent to which inconstancy is heritable rather than just an expression of developmental instability. However, as the developmental and genetic basis of sex determination in plants remain largely unknown, the basis of inconstancy is

also obscure. For only two dioecious species (Persimmon, *Diospyros lotus*, Ebenaceae; Akagi *et al.* 2016; and *Asparagus officinalis*, Asparagaceae; Murase *et al.* 2016) have the sex-determining genes been identified. Work on monoecious melon and cucumber has identified pathways of sex determination of individual flowers and shown that loss-of-function mutations may result in dioecy as well as in the restoration of hermaphroditism (Boualem *et al.* 2015), but whether the same genes are responsible for sex determination in related dioecious lineages is questionable (Ma & Pannell 2016). It is however likely that different and multiple genes are involved in sex determination in the different groups of dioecious species (Tanurdzic & Banks 2004; Diggle *et al.* 2011; Pannell 2017), a hypothesis that has implications not only for the evolution of dioecy (Golenberg & West 2013; Renner 2016), but also for its breakdown.

If sex is determined by the segregation of sex chromosomes with a degenerate Y or W, the breakdown of dioecy will depend on whether selection for increased leakiness occurs on the homogametic or the heterogametic sex, as well as on the age of the sex chromosomes and the extent of degeneration. Surprisingly little is known about sex determination of the vast majority of dioecious species; only about 40 species are known to have sex chromosomes (Westergaard 1958; Ming *et al.* 2011). Much could be learned from a description of the sex-determining systems of any of the many yet ill-studied species. Tools are being developed for the discovery of sex chromosomes through sequencing of nonmodel species (reviewed in Muyle *et al.* 2017), and these could help identify the sex-determining genes that are expected to reside on the sex chromosomes (Ming *et al.* 2011; Charlesworth 2016; Muyle *et al.* 2017). The knowledge of sex-determining systems in families with several groups of dioecious species might also contribute to the better reconstruction of ancestral states: if rather distant species share the same sex chromosomes and sex-determining genes, dioecy is likely to be ancestral. However, this is only possible if the turnover of sex chromosomes is not too frequent, but little is known about this turnover rate at present. The emerging idea that sex chromosomes can be lost and regained (cf. Bachtrog *et al.* 2014; Muyle *et al.* 2017) is yet another point that supports the view that dioecy is probably neither frequently a stable end-point in sexual-system evolution nor a dead end, but a state that may often be lost to the re-evolution of monoecy or hermaphroditism.

Note added in proof

Goldberg *et al.* (2017), using a phylogenetic approach to study sexual system transitions in 40 angiosperm genera, find that

transitions away from dioecy towards monoecy or hermaphroditism are approximately as common as transitions towards dioecy. The authors report large variation between genera in the dominant polarity of transitions between combined and separate sexes, suggesting that different selective forces and mechanisms likely operate in different clades. Their paper stresses a need for more research into the mechanisms of the loss of dioecy, and provides a list of species that probably have lost dioecy in their recent history. Their results and conclusions support the majority of the hypotheses and research directions put forward here.

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