

LETTER

Low siring success of females with an acquired male function illustrates the legacy of sexual dimorphism in constraining the breakdown of dioecy

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Abstract

Dioecy has often broken down in flowering plants, yielding functional hermaphroditism. We reasoned that evolutionary transitions from dioecy to functional hermaphroditism must overcome an inertia of sexual dimorphism, because modified males or females will express the opposite sexual function for which their phenotypes have been optimised. We tested this prediction by assessing the siring success of monoecious individuals of the plant *Mercurialis annua* with an acquired male function but that are phenotypically still female-like. We found that pollen dispersed by female-like monoecious individuals was ~ 1/3 poorer at siring outcrossed offspring than pollen from monoecious individuals with an alternative male-like inflorescence. We conclude that whereas dioecy might evolve from functional hermaphroditism by conferring upon individuals certain benefits of sexual specialisation, reversion from a strategy of separate sexes to one of combined sexes must overcome constraints imposed by the advantages of sexual dimorphism. The breakdown of dioecy must therefore often be limited to situations in which outcrossing cannot be maintained and where selection favours a capacity for inbreeding by functional hermaphrodites.

Keywords

Dioecy, functional hermaphroditism, gain curves, inflorescence architecture, *Mercurialis annua*, monoecy, sex allocation, sexual dimorphism.

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INTRODUCTION

Dioecy is found in about 6% of species, but in about half of all families of flowering plants (Renner & Ricklefs 1995; Weiblen *et al.* 2000; Renner 2014). This distribution might suggest that dioecy is an evolutionary dead end or ‘failure’ (Westergaard 1958; Bull & Charnov 1985; Heilbut 2000), such that lineages with separate sexes diversify less and are more prone to extinction than their hermaphroditic counterparts. However, the dead-end hypothesis has been challenged by analysis suggesting that the evolution of dioecy might actually increase lineage diversification, and that the scattered phylogenetic distribution of dioecious species might be explained by frequent reversions from dioecy to functional hermaphroditism, that is, to a state in which plants have either bisexual flowers or flowers of both sexes (‘monoecy’) (Käfer & Mousset 2014; Käfer *et al.* 2014, 2017; Sabath *et al.* 2015).

Evidence for reversions from dioecy to hermaphroditism or monoecy has been accumulating for some time. Phylogenetic analysis of clades in which both dioecy and functional hermaphroditism occur indicates that dioecy has not always been derived from, but may also be ancestral to, hermaphroditism or monoecy (Goldberg *et al.* 2017; Käfer *et al.* 2017). For instance, dioecy was lost several times in the large and

successful Cucurbitaceae family (Volz & Renner 2008; Schaefer & Renner 2010), as well as in the genera *Bursera* (Becerra & Venable 1999), *Garcinia* (Sweeney 2008), *Gallium* (Soza & Olmstead 2010) and *Dodonaea* (Harrington & Gadek 2010). Within genera, Goldberg *et al.* (2017) found that transitions towards combined sexes were no less common than those towards separate sexes. In many of these reversions, dioecy evolved from monoecy, not hermaphroditism with bisexual flowers, suggesting that the association between dioecy and monoecy (Renner & Ricklefs 1995) may be explained not only by the evolution of dioecy from hermaphroditism via monoecy, but also by the breakdown of dioecy to monoecy.

The breakdown of dioecy presumably involves the selection of individuals with ‘leaky’ sex expression, that is, males producing occasional fruits and seeds, or females producing occasional flowers with functional stamens and pollen. Leaky sex expression is common in dioecious populations (reviewed in Ehlers & Bataillon 2007; Cossard & Pannell 2018), and has been invoked in models for the breakdown of dioecy in both plants (Crossman & Charlesworth 2014) and animals (Pannell 2008). Lloyd (1975a) suggested that dioecy had broken down in *Leptinella* as a result of selection of leaky males following colonisation. Similarly, monoecy in the *Mercurialis annua* species complex probably evolved from ancestral dioecy (Krahenbuhl *et al.* 2002;

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Obbard *et al.* 2006) under selection for reproductive assurance in metapopulations with frequent colonisation (Pannell 2001).

Although the breakdown of dioecy clearly involves changes in sex expression and sex allocation, the relative fitness of males, females and invading hermaphrodites must also depend on the extent to which the dioecious population is sexually dimorphic for non-reproductive traits. Sexual dimorphism is almost ubiquitous in dioecious plants, with males and females differing in morphological, physiological, defence, life-history, resource acquisition and inflorescence traits (Darwin 1871; Geber *et al.* 1999; Fairbairn *et al.* 2007; Moore & Pannell 2011; Barrett & Hough 2013). For instance, females tend to have larger leaves in *Leucadendron* (Bond & Midgley 1988), show a greater photosynthetic capacity in *Salix integra* (Tozawa *et al.* 2009), and are generally less susceptible to herbivory (Cornelissen & Stiling 2005). The evolution of sexual dimorphism likely allows males and females in dioecious populations to express phenotypes that enhance siring success and seed production, respectively, but that might compromise these functions if expressed in the other sex (Lande 1980; Cox & Calsbeek 2009). Yet reversion to functional hermaphroditism must bring about just this sort of compromise, because modified males or females will express their newly acquired function in the context of a phenotype optimised for the opposite sex. The evolution of sexual dimorphism should constrain the breakdown of dioecy in a way that goes beyond a simple sex-allocation trade-off.

The likely constraints of sexual dimorphism on the breakdown of dioecy are well illustrated in wind-pollinated species, in which male and female inflorescences are often quite different (Lloyd & Webb 1977; Whitehead & Real 1983; Weberling 1992; Galonka *et al.* 2005; Friedman & Barrett 2009b; Harris & Pannell 2010; Harder & Prusinkiewicz 2013). Male (or staminate) flowers of wind-pollinated species are typically held on flexible stalks or 'peduncles', which facilitate pollen liberation from anthers and pollen dispersal by wind (reviewed in Harder & Prusinkiewicz 2013). In trees, these structures often hang from the branches. In herbs, they are typically held above the plant canopy. In both situations, pollen is liberated when turbulent gusts shake male flowers or anthers (Urzay *et al.* 2009). In contrast, female (or pistillate) flowers are typically held on more rigid inflorescences, and pollen is picked up by stigmas as they impact their surfaces, or from non-viscous eddies around the flower (but see Cresswell *et al.* 2010), whereas the two sexual functions of bisexual flowers will often interfere with one another in wind-pollinated plants, compromising fitness (Friedman & Barrett 2009b; Harder & Prusinkiewicz 2013), the inflorescences of plants with separate male or female flowers (in dioecious or monoecious species) may be optimised for each sex separately (Friedman & Barrett 2008), for example, male flowers of *Zea mays* are born on flexible tassels at the shoot apex, whereas female flowers develop in erect ears in the leaf axils (Aylor *et al.* 2003).

The male and female inflorescences of the plant *M. annua* L. (Euphorbiaceae) illustrate the divergent strategies for the two sexes of wind-pollinated herbs and suggest how they might constrain the breakdown of dioecy (Fig. 1a). *Mercurialis annua* is a complex of European ruderal plants that vary in their sexual systems and inflorescences (Durand 1963;

Durand & Durand 1992; Pannell *et al.* 2008). Dioecy is ancestral in *Mercurialis* and is widespread in *M. annua* in Europe, but monoecy has apparently evolved from dioecy in the Iberian Peninsula and north Africa through the spread of leaky females with an enhanced male function (Obbard *et al.* 2006). In dioecious populations, males disperse their pollen from staminate flowers on 'pedunculate' stalks held above the foliage, whereas females produce their flowers on subsessile pedicels in leaf axils (Eppley & Pannell 2007). The male function of the monoecious *M. annua* is associated with a female phenotype that differs from males in terms of life-history, nitrogen budget and allocation to defence (Hesse & Pannell 2011b, c; Sanchez-Vilas & Pannell 2011a,b; Sanchez-Vilas *et al.* 2011; Labouche & Pannell 2016; Tonnabel *et al.* 2017). Importantly, monoecious individuals hold both their male and female flowers in leaf axils, whereas males place flowers on erect inflorescence stalks ('peduncles') (Fig. 1). Pollen dispersed from male peduncles is a 60% better at siring outcrossed progeny than that from monoecious inflorescences (Eppley & Pannell 2007). We might view the poor pollen dispersal of monoecious individuals with an inflorescence morphology derived from females as a legacy of sexual dimorphism that constrains the breakdown of dioecy in *M. annua*.

Here, we compare the siring success of the typical monoecious individuals with female-like ('F-like') inflorescences with that of hitherto undescribed monoecious individuals of *M. annua* that have longer inflorescences similar to those of males ('M-like'). Populations of the F-like form are widespread around the coast of the Iberian Peninsula, whereas populations of the M-like form are restricted to southern and eastern Spain. Although the two forms rarely co-occur, their distributions are broadly sympatric. The evolutionary paths linking dioecy to the two monoecious forms in *M. annua* are not well understood. However, pedunculate inflorescences are associated with a Y-linked marker in all lineages that have them, except in M-like monoecious individuals (unpubl. data). It is thus likely that peduncles of the M-like lineage are not derived directly from males and have evolved independently. Either way, the peduncle represents a potential improvement on the F-like monoecious inflorescence that is likely derived from females.

We hypothesised that the M-like form should enjoy greater siring success than the F-like form, in competition both with males and with the F-like form. We first compared inflorescence morphology and pollen production of males with those of M-like and F-like monoecious forms, then tested our hypotheses by evaluating siring success of the three phenotypes in common gardens. We also tested for trade-offs between male and female allocation within and among populations. Our results support the notion that monoecious individuals that retain an inflorescence morphology derived from females are indeed poorer at siring progeny than those that combine monoecy with male-like inflorescences, illustrating the constraint posed by sexual dimorphism in the breakdown of dioecy. Our analysis also suggests that transitions from dioecy to functional hermaphroditism are likely to be associated with a shift from obligate outbreeding to facultative inbreeding, as implied by models of the breakdown of dioecy (Maurice & Fleming 1995; Wolf & Takebayashi 2004; Ehlers & Bataillon 2007; Pannell 2008; Crossman & Charlesworth 2014).

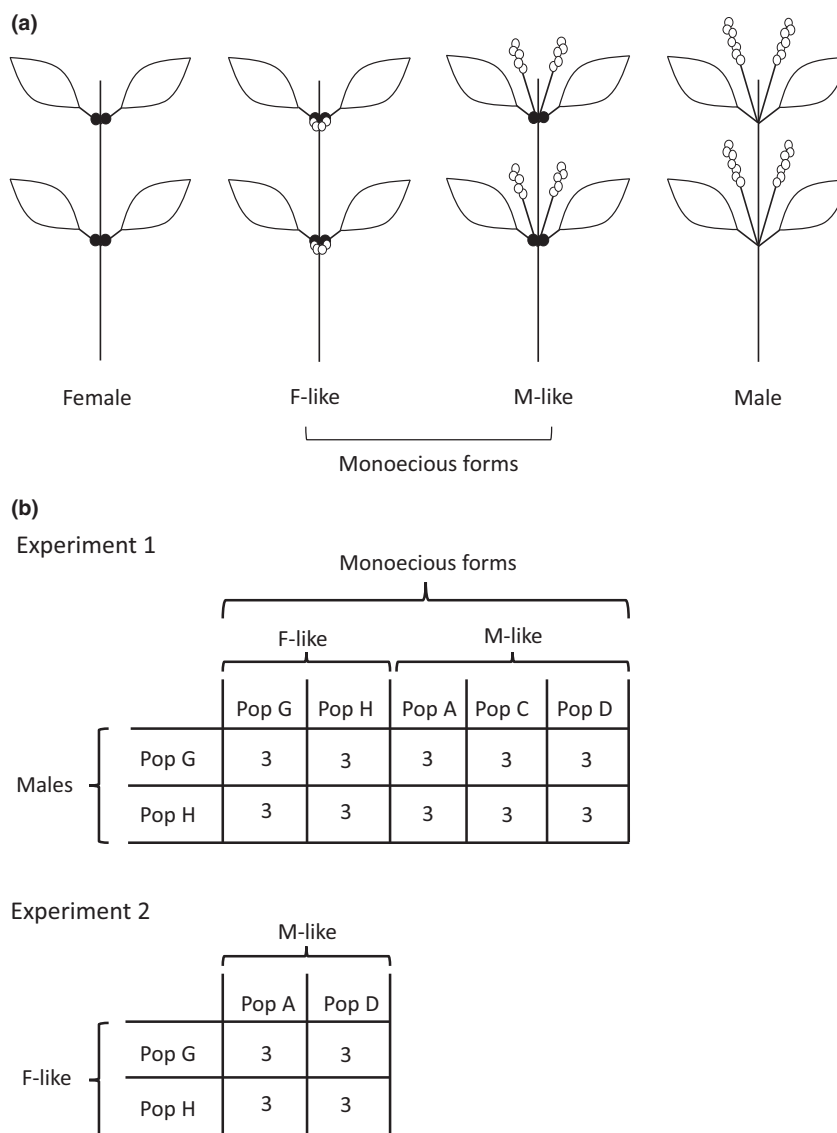


Figure 1 (a) Cartoon drawings illustrating the inflorescence variation in *Mercurialis annua* between females, F-like monoecious individuals, M-like monoecious individuals, and males. Black circles represent female flowers or fruits. White circles represent male flowers. Note that: peduncles of males are depicted as being longer than those of M-like monoecious individuals, which are in turn longer than those of F-like individuals; and males are depicted as producing more male flowers than M-like monoecious individuals (see Results). (b) Experimental design for Experiments 1 and 2. There were three replicates for each combination of competing populations indicated. In Experiment 1, males from each of two androdioecious populations competed either against female-like monoecious individuals from each of the same two populations, or male-like monoecious individuals from each of three populations. In Experiment 2, female-like monoecious individuals from each of two of their populations competed against male-like monoecious individuals from each of two of their populations.

MATERIALS AND METHODS

Study populations, seed collection, and seedling establishment

We collected seed from about 30 seed-producing individuals from each of five populations: two androdioecious populations in which males co-occurred at frequencies of approximately 30% with F-like monoecious individuals (populations G and H); and three monoecious populations with only M-like monoecious individuals (populations A, C and D). We sowed seeds in bulk in seedling trays and transplanted seedlings (7–10 days after sowing) individually into small pots. When their sex could be determined (at 21 days), we re-

transplanted them into 15 cm diameter pots and established them in their mating arrays outside.

Experimental design

We conducted two experiments to compare male and female components of fitness: in Experiment 1 (conducted at Wytham field station, University of Oxford), each monoecious form competed separately against males; in Experiment 2 (conducted at the University of Lausanne), we competed the two monoecious forms against one another (Fig. 1b). For Experiment 1, we placed males from each androdioecious population with individuals of either the M-like and F-like monoecious

forms, yielding ten different common-garden combinations. Each combination was replicated three times (30 mating arrays in total), with seven males and 42 monoecious individuals arranged so that males occurred only once in each row and column. Plants were allowed to mate with one another for 4–6 weeks, when they were harvested, with seeds collected from each mother separately (Note that any seeds sired prior to establishment of the respective arrays had already dispersed, so that we can be sure that all progeny harvested were sired under the treatment conditions.)

In Experiment 2, mating arrays comprised individuals of both monoecious forms together. We used genotypes from two F-like (G and H) and M-like populations (A and D), competing in all four combinations (A–G, A–H, D–G and D–H), with three replicates each (twelve arrays in total). Arrays were established as squares with 25 F-like alternating with 24 M-like individuals. Plants were harvested after 6 weeks.

In both experiments, arrays were established across the available area. Arrays for Experiment 1 were tens of metres apart, but for Experiment 2 they had to be placed at approximately three metres from each other. To prevent immigration of pollen from adjacent arrays, we erected plastic barriers 1.5 m tall between them. A similar setup prevented gene flow among contiguous plots in a previous experiment (Dorken & Pannell 2009). Any gene flow among arrays would have reduced experimental power, so our results are conservative. The barriers between arrays likely also reduced wind-flow within the arrays, and may also have affected the mating system somewhat, but we do not expect this effect to have been large; see Discussion.

Data collection

Individuals in the outer edge of all arrays, except males in Experiment 1, were excluded from analysis. Experiment 1 had a final sample size of 851 plants (278 F-like, 373 M-like, and 210 male individuals); Experiment 2 had a final sample size of 300 plants (156 F-like and 144 M-like individuals). We measured the height of all plants, disregarding the additional height of pedunculate inflorescences. For a subsample of seven plants of each inflorescence form and array, we measured the length of five randomly chosen inflorescences (both experiments), and the biomass of all staminate flowers. Previous work has shown that pollen biomass is strongly correlated with the biomass of staminate flowers (Pannell 1997b,c). All plants from both experiments were allowed to dry slowly and release their seeds in porous bags. We weighed the seeds of each plant together, as well as the aboveground plant biomass.

Assessment of relative siring success

For Experiment 1, we used the sex ratio in the progeny to estimate the relative siring success of males and monoecious individuals. In *M. annua*, males are determined by the expression of a dominant allele at a single locus, so that males are heterozygous (i.e. XY) (Russell & Pannell 2015; Veltsos *et al.* 2018). Progeny sired by males will thus be 50% male and 50% monoecious. Accordingly, we calculated the relative siring success of males in each array as twice the frequency of

male progeny, based on 200 progenies grown to sexual maturity per array (6000 individuals in total).

For Experiment 2, we used microsatellites to estimate selfing rates on and siring success by F-like and M-like individuals. We genotyped 30 plants from progeny produced by each of the two monoecious forms from each of the twelve replicate arrays (total of 720 plants), using DNA from young silica-dried leaves. DNA was extracted with a BioSprint 96 robot (Qiagen, Hilden, Germany), using a BioSprint 96 DNA plant kit (Qiagen). Individuals were scored for five microsatellite markers (Mh14, Mh15, Mh19, Mh52 and Mh91(2)) that provide good separation between the monoecious populations sampled (Korbecka *et al.* 2010). All five markers were amplified in a single multiplexed PCR reaction, following the protocol described in Korbecka *et al.* (2010). We processed the samples in an ABI 3100 sequencer (Applied Biosystems, Foster City, CA, USA), and analysed the results with GeneMapper v.4.0 (Applied Biosystems). Individual genotypes were classified as having been sired by a father of the same or a competing phenotype in the array; resolution was insufficient to assign paternity to specific individuals. For populations of the F-like form, we estimated selfing rates using the software RMES (David *et al.* 2007); those of the M-like form had almost no genetic variability.

Data analysis

We used mixed models to analyse: plant height; plant biomass; mean length of peduncles per plant; pollen biomass and seed biomass; sex ratio in the progeny of Experiment 1; and, for Experiment 2, the proportion of progeny of parents with different phenotypes. As our primary interest was to determine the functional effect of two contrasting monoecious inflorescence forms in *M. annua*, we defined inflorescence form as a fixed variable, and population within inflorescence form and array as random variables. We tested for a trade-off between male and female reproduction of monoecious individuals in Experiment 1 by fitting a model with pollen mass as the response variable and biomass of seeds as the independent variable, including biomass as a covariate to allow assessment of the male and female reproductive allocation in relation to size. Population and array were included as random terms in the model.

We used Gaussian models for all variables except for the sex ratio and interform crossing rate, for which we used a binomial model. Data were log-transformed when necessary to normalise residuals. All data analysis was implemented in R v.3.3.2 (R Development Core Team 2016) using package lme4 (Bates *et al.* 2015). Significance of fixed effects and differences between inflorescence forms were evaluated using the package lmerTest (Kuznetsova *et al.* 2017), or z-tests in the case of ratios. Significance of random effects was assessed by likelihood-ratio tests.

RESULTS

Phenotypic variation among inflorescence forms

Phenotypic measurements of the three phenotypes (males, F-like and M-like individuals) were broadly consistent across

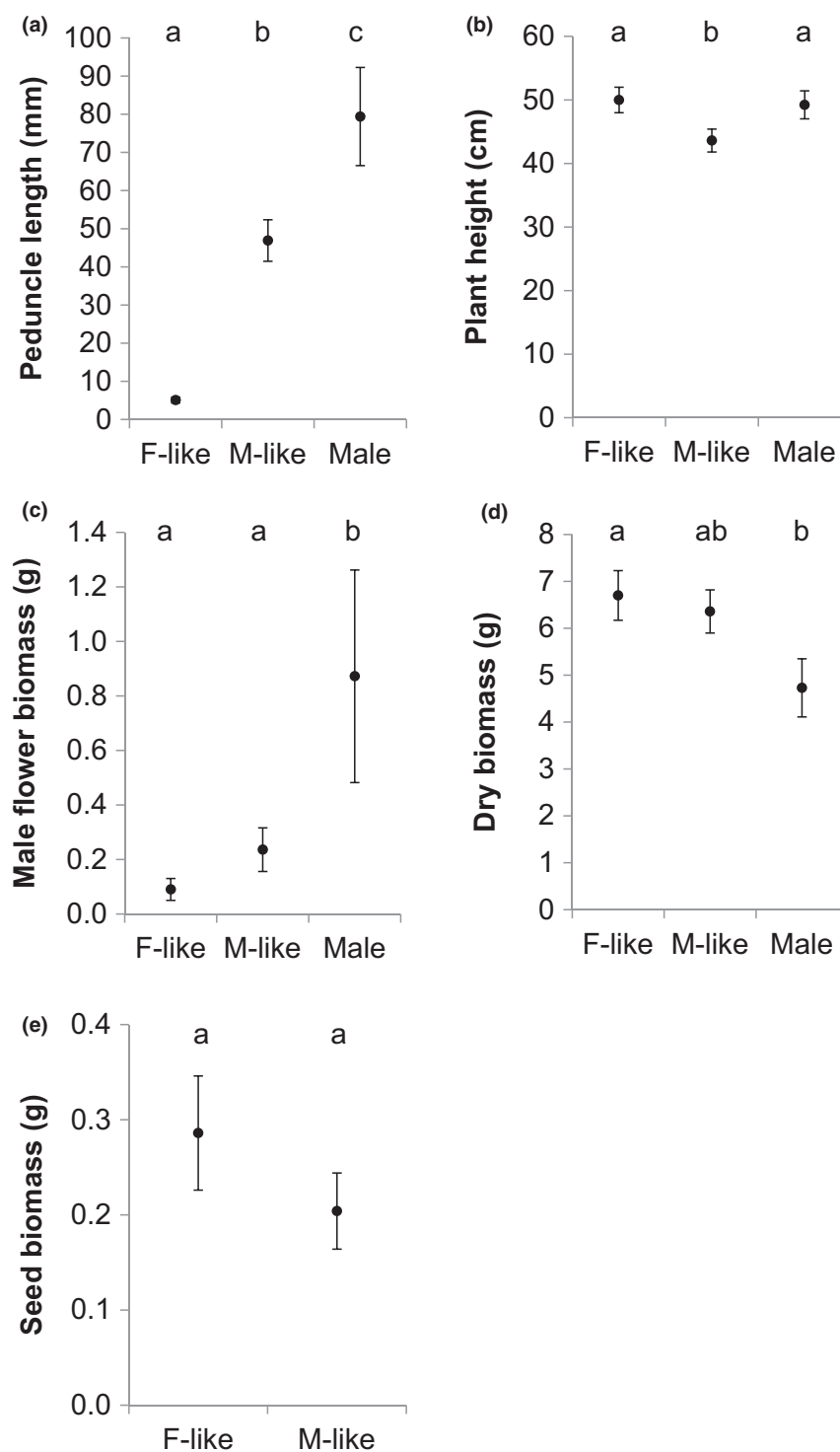


Figure 2 Mean values for several size and allocation traits for males, female-like (F-like) and male-like individuals (M-like) of *Mercurialis annua*: (a) height including inflorescence; (b) dry biomass; (c) male flower biomass; (d) total biomass of seeds produced; (e) peduncle length. Measurements were taken from mating arrays in Experiment 1 under uniform environmental conditions. Error bars show the standard error. Significant pairwise differences ($P < 0.05$) are indicated by different low case letters. Sample sizes for panels (b), (d) and (e): males = 210, F-like = 278, M-like = 373. Sample sizes for panels (a) and (c): males = 210, F-like = 84, M-like = 126.

both Experiments 1 and 2; for brevity, we therefore report measurements for Experiment 1 in the text (Fig. 2) and present all data for both experiments in the Supplementary Materials (Tables S1 and S2).

Peduncle length differed between all three phenotypes: peduncles of males were longer than those of M-like monoecious individuals, which were much longer than those of F-like individuals (Fig. 2a and Table S1). F-like monoecious

individuals were similar in height to males, but significantly taller than M-like monoecious individuals (Fig. 2b and Table S1). Males invested much more in male flower production than did both monoecious forms (Fig. 2c). Even though there were substantial differences in both vegetative and reproductive traits at the population level (biomass: $\chi^2_1 = 30.6$, $P < 0.001$; seed biomass: $\chi^2_1 = 3.47$, $P = 0.062$; male flower biomass: $\chi^2_1 = 51.2$, $P < 0.001$; Tables S1 and S2), large within-form population variation and low population number rendered differences among forms non-significant (biomass: $t_{1.06} = 0.50$, Fig. 2d; $P = 0.30$; seed biomass: $t_{16} = 1.40$, Fig. 2e; $P = 0.18$; pollen biomass: $t_4 = 1.99$, $P = 0.12$, Fig. 2c).

Inferred siring success

In Experiment 1, in which one or other of the two monoecious forms co-occurred with males, there were substantially more monoecious progeny (as opposed to males) from arrays with M-like (proportion = 0.92 ± 0.02) than with F-like individuals (proportion = 0.68 ± 0.05 ; $z = 6.22$, $P < 0.001$; Fig. 3a). Given that maleness in *M. annua* is determined by a dominant allele (see Materials and Methods), we thus inferred that M-like individuals sired 35% more progeny when competing with males than F-like individuals did. Equivalently, F-like individuals sired 25% fewer progeny than M-like individuals sired.

In Experiment 2, in which individuals of the two monoecious forms co-occurred, M-like individuals sired more than three times more seeds than F-like individuals did ($z = 9.3$, $P < 0.001$; Fig. 3b). Of progeny with F-like individuals as both parents, 35% were self-fertilised. Taking into account progeny of crosses between the two forms, F-like mothers thus self-fertilised a fraction 0.15 of their progeny (Table S3). There was insufficient variation at the microsatellite loci to estimate the selfing rate of M-like individuals. However, if we assume a similar selfing rate for both forms (see Discussion), we may infer that pollen dispersed by F-like individuals in Experiment 2 sired 31% fewer outcrossed progeny than pollen dispersed by M-like individuals.

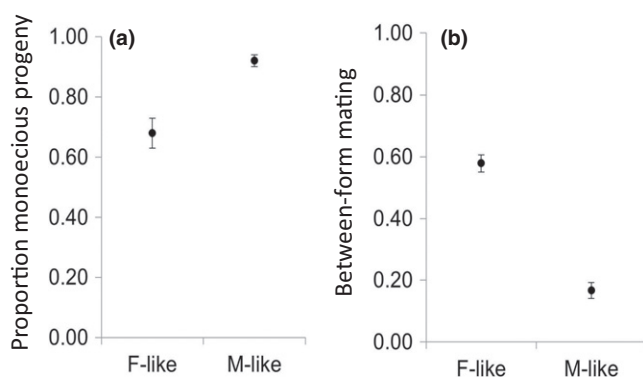


Figure 3 (a) Mean proportion of males in the progeny of female-like (F-like) and male-like (M-like) monoecious individuals grown in mating arrays together with males (Experiment 1). (b) Proportion of progeny of female-like and male-like monoecious individuals attributable to crosses with the other inflorescence form after mating in mixed mating arrays in Experiment 2. Error bars show the standard error. Samples sizes: (a) M-like = 3600, F-like = 2400; (b) M-like = 360, F-like = 360.

Trade-off between male and female reproduction

There was a significant trade-off between male and female allocation to reproduction within populations ($t_{4.3} = 3.47$, $P = 0.023$; Fig. 4), with significant variation in the strength of the trade-off among populations ($\chi^2_3 = 22.2$, $P < 0.001$; Fig. 4). Biomass ($t_{184} = 7.04$, $P < 0.001$) and M-like inflorescence type ($t_{10.1} = 2.39$, $P = 0.038$) had a positive and significant contribution to the model, that is, individuals with higher biomass and peduncles allocated more to male function for a given female allocation.

DISCUSSION

High siring success of M-like monoecious individuals

Monoecy in *M. annua* has evolved from dioecy via the modification of females that produce male flowers, and that disperse pollen from sub-sessile axillary inflorescences similar to those of females. Our results support the hypothesis that this female-like (F-like) inflorescence morphology, a legacy of the breakdown of dioecy, compromises the siring success of monoecious individuals in comparison with that of plants with a male-like (M-like) pedunculate inflorescence. Monoecious individuals with short F-like inflorescences sired about 25% fewer progeny in mating arrays with males than the newly characterised form with long M-like inflorescences that occurs in part of the species' range, and they sired 31% fewer progeny when in direct competition with the M-like form. These results are coherent with those of Eppley & Pannell (2007), who found that pedunculate inflorescences conferred a substantial siring advantage per pollen grain on males compared with F-like individuals lacking peduncles. The fact that, in our experiments here, the siring success of the M-like form was higher than that of F-like form but lower than that of males is consistent with their dispersal of pollen from peduncles of intermediate length.

Our siring estimates assumed that M-like and F-like forms have the same selfing rate. Individuals of the F-like form self-fertilised a proportion 0.15 of their seeds. This relatively low value is consistent with the low selfing rates of F-like monoecious *M. annua* in both dense experimental and field populations (Eppley & Pannell 2007; Korbecka *et al.* 2011), and it suggests that the erection of barriers around the arrays to prevent between-array pollen movement probably did not affect the mating system within the arrays very much. The result is also consistent with the presence of males in androdioecious populations of *M. annua*, because males cannot be maintained with hermaphrodites or monoecious individuals that self-fertilise a large proportion of their progeny (Lloyd 1975b; Charlesworth & Charlesworth 1978; Charlesworth 1984). On the one hand, M-like individuals might be less likely to fertilise their own ovules than F-like individuals, because they disperse their pollen better. On the other hand, M-like individuals show a tendency to produce more pollen than F-like individuals (though the difference was not significant), which might increase their selfing rate (because selfing rates correlate with pollen production in *M. annua* more generally; unpublished data). Given these likely opposing effects on the selfing rate of the

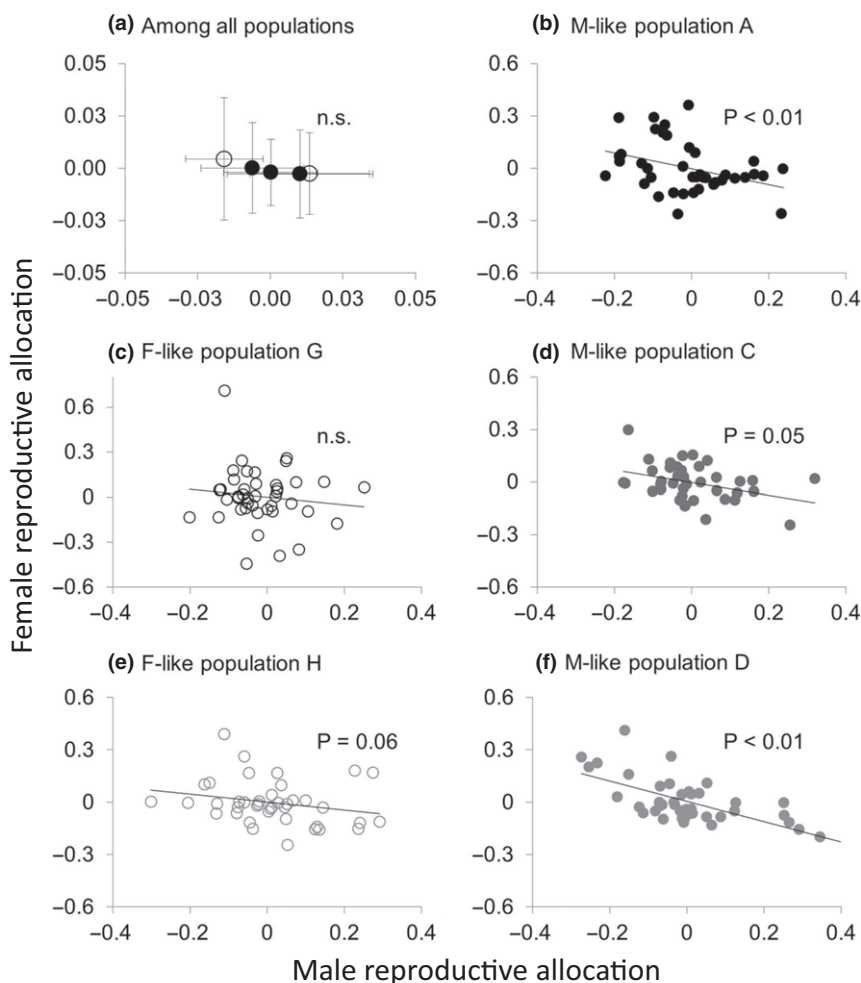


Figure 4 Trade-off between male and female allocation at the individual plant and population level from Experiment 1. (a) Male vs. female average relative reproductive allocation at the population level. Hollow circles represent F-like populations; solid circles represent M-like populations. (b), (d) and (f) Male and female relative reproductive allocation at the individual level in each of three populations with M-like individuals (populations A, C and D, respectively). (c) and (e) male and female relative reproductive allocation at the individual level in two F-like populations (populations G and H, respectively). Relative reproductive allocation is represented here as the residuals of male and female reproductive investment after removing the effect of total biomass.

reproductive strategy of M-like individuals, it seems plausible that the selfing rates of the two forms were similar in our arrays.

The higher siring success of M-like monoecious individuals of *M. annua* in experimental mating arrays helps to explain why they rarely co-occur with males (c.f., Pannell *et al.* 2014), as well as why the two monoecious forms rarely co-occur. Although the weak trade-off among monoecious individuals between their male and female functions might allow co-existence of the two forms (with one emphasising male function and the other emphasising female function), the high fitness of M-like individuals means that F-like individuals should be maintained at low frequencies and could more easily be lost by drift, especially because population size fluctuates so much (Dorken *et al.* 2017). Similarly, although males produce more pollen and have longer peduncles than M-like individuals, our Experiment 1 showed that the siring success of the latter is sufficient to keep males at low frequency; they, too, might thus easily be lost by drift. The effect of drift and

demographic stochasticity has similarly been shown to allow the loss of style morphs of *Eichhornia paniculata* that are maintained by negative frequency-dependent selection in large populations (Barrett *et al.* 1989).

The high siring success of the M-like monoecious form of *M. annua* also raises the question of why it has not spread more widely across the Iberian Peninsula, replacing the F-like form. It is possible that the superior mating strategy of the M-like form is costly in ways we have not evaluated, for example, in terms of physiological and life-history traits that are sexually dimorphic in dioecious or androdioecious *M. annua* (Hesse & Pannell 2011c; Sanchez-Vilas & Pannell 2011b; Sanchez-Vilas *et al.* 2011; Labouche & Pannell 2016; Tonnabel *et al.* 2017). For example, the F-like form might enjoy an advantage over the M-like form during periods of colonisation (e.g. because it confers greater reproductive assurance, Friedman & Barrett 2009a). Such an explanation would be consistent with the metapopulation model proposed by Pannell (1997a), notably if a greater seed production by

the F-like form allowed it to establish more viable demes early after colonisation (Pannell 2001). Estimates of relative seed production and progeny performance from a wider sample of populations would help to evaluate this possibility.

Sexual dimorphism and the stabilisation of dioecy

Variation in inflorescence morphology in *M. annua* illustrates how sexual dimorphism might stabilise the maintenance of separate sexes in dioecious plants. Sex-allocation theory predicts that if either the male or the female sexual function (or both functions together) have accelerating fitness gain curves, then the 'fitness set' is concave, and dioecy should be evolutionarily stable (Charnov *et al.* 1976; Charnov 1982; West 2009). Sexual dimorphism likely allows unisexual individuals to perform better than bisexual individuals in their corresponding sexual function, which should enhance the concavity of the fitness set (Fig. 5). Due to lack of specialised structures for pollen dispersal in F-like monoecious individuals of *M. annua*, their male fitness gain curve is probably saturating. In contrast, our results suggest that the superior inflorescence structure of the M-like form likely diminish this saturating tendency. (Saturation of female fitness gain curves likely remains unchanged.) If so, we suggest that the fitness set of the M-like form should relax conditions for their invasion into

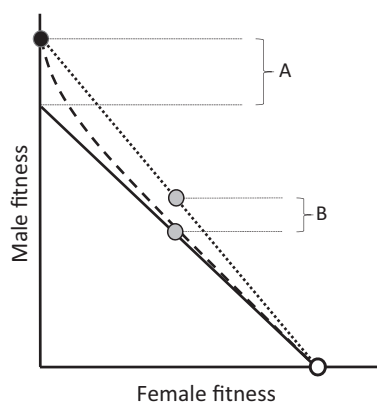


Figure 5 Heuristic schema illustrating the hypothesised influence of the adoption of a specialised male-like inflorescence by males or functional hermaphrodites on the fitness set relating male to female components of fitness. The unbroken diagonal straight line depicts a linear fitness set representing a simple trade-off between male and female functions of a wind-pollinated plant like *Mercurialis annua*, in the absence of any sexual specialisation by males or females. Females (open white circle) occupy the extreme female end of this line. Female-like individuals allocating resources to pollen would occupy positions along the straight-line diagonal. Adoption of a specialised male inflorescence increases the male component of fitness (closed black circle), so that males have a fitness greater than that achievable simply by allocating all reproductive resources to male function (represented by the interval A). The adoption by males of the specialised inflorescence effectively renders the fitness set concave (dashed curve). Adoption by functional hermaphrodites of a specialised male-like inflorescence increases their male fitness component above that achievable by female-like functional hermaphrodites (interval B), effectively removing the concavity of the fitness set (dotted line). In our experiments, intervals A and B increased male fitness by about 60 and 45% above the straight-line diagonal. See text for details.

a dioecious population and a transition to functional hermaphroditism, particularly under intense competition for outcross siring (Pannell 2001).

Figure 6 sets out a path that might commonly be followed in plants when dioecy breaks down. In populations that have recently evolved dioecy (e.g. in response to selection for outcrossing; Fig. 6a), the fitness of males and females will be compromised by the expression of genes in individuals of one sex that are better suited to performance of the other, or genes underpinning a reproductive, physiological, life-history, or defence strategy that is not optimised for its own sex. Over time, selection should act to reduce trait correlations between males and females (Lande 1980), optimising male and female phenotypes differently in a sexually dimorphic population (Fig. 6b). It is also possible that selection for sexual specialisation sometimes coincides with, rather than follows, the transition from combined to separate sexes (Willson 1979; Bawa 1980; Givnish 1982) – although the hypothesis is controversial (Thomson & Barrett 1981; Charlesworth 1985). Either way, males and females of dioecious populations end up with phenotypes that enhance their own sex function, but that might not do so for the other, and might indeed be deleterious (Connallon & Clark 2014).

The frequent transitions from dioecy to functional hermaphroditism in flowering plants (Kafer *et al.* 2014, 2017; Goldberg *et al.* 2017) presumably begin with selection of males or females with a leaky sexual function, and the derived hermaphrodites (or monoecious individuals) should inherit the secondary sexual phenotype associated with their ancestral sexual function, male or female (Fig. 6c). Our results suggest that this ancestral phenotype is antagonistic to the newly acquired function in F-like monoecious *M. annua*, and similar reasoning may hold for transitions from separate to combined sexes more generally. For example, many dioecious lineages might revert to functional hermaphroditism via gynodioecy through the selection of leaky males, which are much more common than leaky females (Ehlers & Bataillon 2007; Cossard & Pannell 2018). In these lineages, functional hermaphrodites derived from leaky males will tend to express their acquired female function in the context of floral, physiological, life-history or defence traits that have been selected to optimise siring success, not seed or fruit production. With time, selection should mould the functional hermaphroditic phenotypes in ways that overcome this constraint, finding solutions that optimise both male and female functions and, for example, avoiding their interference (Fig. 6d).

The above reasoning implies that sexual dimorphism in plants should constrain the breakdown of dioecy when outcrossing is favoured by establishing populations with a concave fitness set that become more resistant to the invasion of functional hermaphrodites (Fig. 5). Indeed, once sexual dimorphism has evolved, it is difficult to imagine what could allow a transition from separate to combined sexes without an accompanying change in the mating system. In the case of *M. annua*, monoecy likely evolved from dioecy as a selfing mechanism in response to selection for reproductive assurance in sparse populations (Hesse & Pannell 2011a; Labouche *et al.*

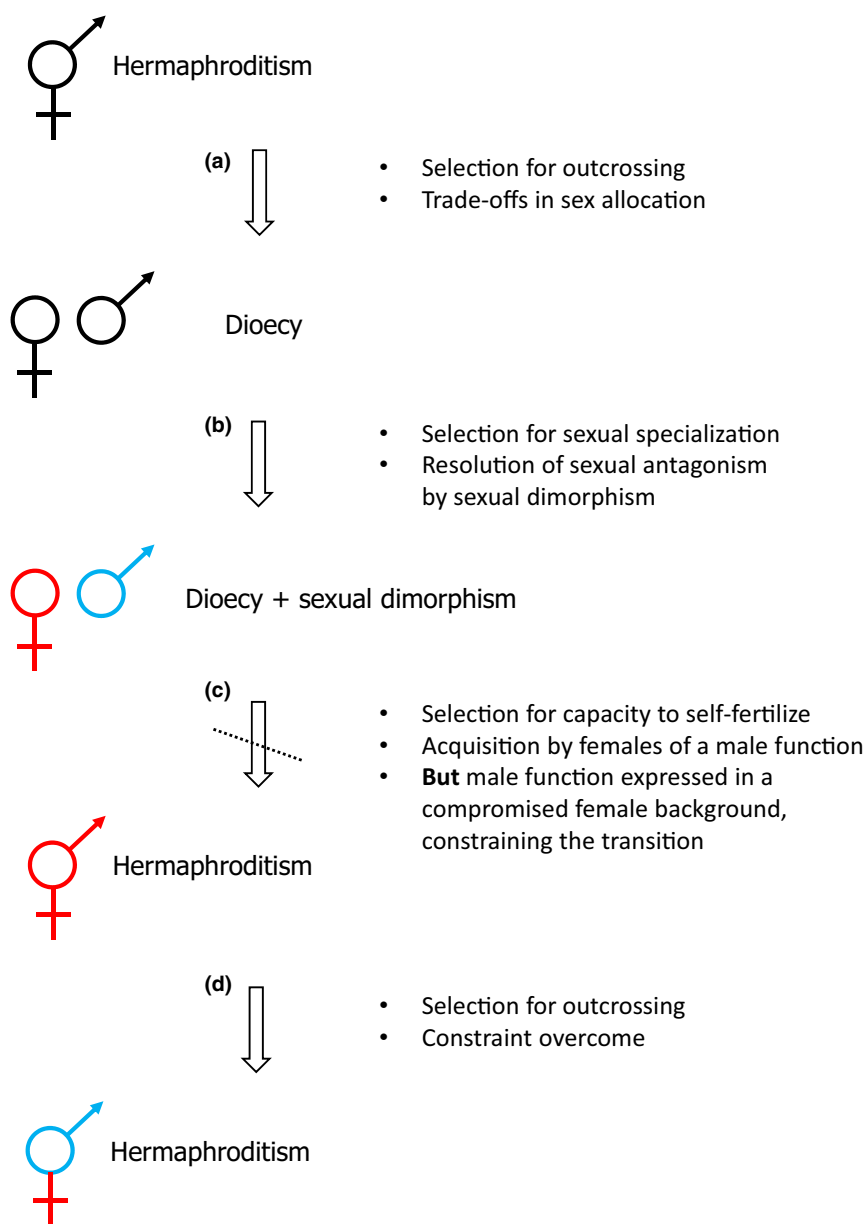


Figure 6 General evolutionary path envisaged for the breakdown of dioecy in flowering plants. (a) Dioecy evolved from hermaphroditism in response to selection to avoid inbreeding, for example via the spread of male and female sterility mutations and increased allocation to the remaining sexual function. (b) Over the course of its persistence, the dioecious population evolves secondary sexual dimorphism, with males and females expressing phenotypes that are optimised for their respective sexual functions. (c) Dioecy breaks down in response to selection for reproductive assurance and a capacity to self-fertilise by individuals with leaky sex expression. The functional hermaphrodites thus formed must express their newly acquired sexual function in the context of a phenotype optimised for the other sexual function, compromising the fitness of derived functional hermaphrodites and constraining the breakdown of dioecy. (d) With time, selection for improved reproductive performance through both sexual functions moulds the hermaphroditic strategy, finding phenotypes that improve one sexual function without, or with diminished, deleterious effects to the other.

2017), during episodes of range expansion (Pujol & Pannell 2008; Pujol *et al.* 2009), or during colonisation of disturbed habitat in metapopulations (Pannell 2001; Pannell & Dorken 2006; Pannell *et al.* 2008). Similar processes of mate limitation have been invoked for the breakdown of dioecy in other plant and animal lineages (Baker & Cox 1984; Charlesworth 1993; Maurice & Fleming 1995; Pannell 2002; Wolf & Takebayashi 2004; Ehlers & Bataillon 2007; Crossman & Charlesworth 2014).

CONCLUSION

The broad variation in sexual systems presented by *M. annua* has hitherto allowed it to be used to test a range of general hypotheses concerning transitions between, and the maintenance of, combined vs. separate sexes in plants. Previous work has focused on differences in sex allocation between gender strategies to understand the distribution of sexual systems across the species' range (e.g. Pannell *et al.* 2014). Other

studies have explored reasons and implications for the evolution of sexual dimorphism in populations in which males co-occur with either females or hermaphrodites, drawing attention to the likely importance of differences in the relative costs of reproduction between males and females (e.g. Tonnabel *et al.* 2017; and references cited therein). The variation in inflorescence morphology among contrasting hermaphrodites in the species complex has now exposed the possibility that when dioecy breaks down, the initial fitness of the new combined-sex phenotype will be compromised by a history of selection under dioecy, as set out in Fig. 6.

The constraints illustrated by our study should apply to any species in which dioecy has become associated with dimorphism in secondary sexual characters that enhance the fitness of gender specialists. Thus, whenever hermaphroditism evolves from dioecy in response to selection for reproductive assurance, the benefits acquired through a capacity to self-fertilise must be greater than the sometimes substantial benefits that males and females derive from sexual specialisation. An important implication is that the evolution of sexual dimorphism should tend to resist such transitions in outcrossing populations. The breakdown of dioecy therefore seems most likely to occur in association with a transition from obligate outcrossing to at least facultative selfing. Models that contrast the fitness gain curves of combined vs. separate sexes must thus account for inbreeding, too (Charlesworth & Charlesworth 1981).

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AUTHORSHIP

LS and JRP conceived the study. LS and ET carried out the experiments. LS conducted all analysis and made the figures. JRP wrote the manuscript with help from LS. All authors commented and approved the manuscript for submission.

DATA ACCESSIBILITY STATEMENT

Data available from the Dryad Digital Repository: <http://doi.org/10.5061/dryad.74hp1kt>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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