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GENDER VARIATION AND TRANSITIONS BETWEEN SEXUAL SYSTEMS IN *MERCURIALIS ANNUA* (EUPHORBIACEAE)

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Evolutionary transitions between hermaphroditism and dioecy have occurred numerous times in the land plants. We briefly review the factors thought to be responsible for these transitions, and we provide a synthesis of what has been learned from recent studies of the annual herb *Mercurialis annua*, in which dioecy (males and females), monoecy (functional hermaphrodites), and androdioecy (males and hermaphrodites) occur in different parts of its geographic range. Previous research on *M. annua* has revealed the importance of genome duplication and hybridization in the origin of much of the observed variation. Here we show, however, that spatial transitions in the sexual system also occur within the same ploidy level. In particular, we present an analysis, using flow cytometry data, of ploidy variation across a previously unstudied transition between hermaphroditism and androdioecy, in which we find that the sexual-system transition is uncoupled from the shift in ploidy levels. We review recent research that shows that such transitions between sexual systems in *M. annua* are consistent with differential selection at the regional level for reproductive assurance during colonization. We also present new experimental data that highlight both the importance of the resource status of plants and that of their local mating context in regulating gender strategies and sex ratios. The studies reviewed and the new results presented emphasize the role that shifts in the ecological and genetic context of plant populations may play in causing transitions between sexual systems.

Keywords: androdioecy, dioecy, gynodioecy, hermaphroditism, mating system, monoecy.

Introduction

By far, the majority of flowering plants are hermaphroditic (Yampolsky and Yampolsky 1922; Sakai and Weller 1999), but transitions between hermaphroditism and dioecy have been frequent (Renner and Ricklefs 1995; Weiblen et al. 2000; Vamasi et al. 2003; Case et al. 2008). Most research has been directed toward understanding shifts from hermaphroditism toward dioecy, with a range of hypothesized evolutionary paths invoked (reviewed by Charlesworth [1999]; Webb [1999]). Indeed, Darwin (1877) set the stage for much of this work by noting the substantial advantages of hermaphroditism and asking why “hermaphrodite plants should ever have been rendered dioecious” (p. 279). In contrast, we might similarly ask why males and females, which may enjoy advantages of gender specialization, should ever be replaced by hermaphrodites. Although probably less frequent than transitions from hermaphroditism to dioecy, the breakdown of dioecy toward hermaphroditism is known to have occurred in both plants and animals (Desfeux et al. 1996; Wolf et al. 2001; Kiontke et al. 2004; Weeks et al. 2006), and a growing body of ideas exists on when such shifts might occur (Charnov et al. 1976; Charnov 1982; Maurice and Fleming 1995; Wolf and Takebayashi 2004). Nevertheless, the empiri-

cal foundation for understanding transitions from dioecy to hermaphroditism remains weak.

From a theoretical point of view, the breakdown of dioecy requires the invasion and spread in a population of either pollen-producing females or seed-producing males. Initially, the spread of hermaphrodites in a dioecious population will yield “trioecy,” a sexual system in which all three gender classes are maintained together, but the conditions under which such a gender trimorphism can be maintained evolutionarily appear to be rather limited (Maurice and Fleming 1995; Wolf and Takebayashi 2004). More likely is the rapid displacement of either the females or the males from the population, with the invading hermaphrodites maintained in an androdioecious or a gynodioecious population, respectively. Although gynodioecy is understood to be an important step in the evolution of dioecy from hermaphroditism, it is not known to have played a major role in the breakdown of dioecy. In contrast, while androdioecy is exceedingly rare in absolute terms (Darwin 1877; Charlesworth 1984), almost all the known androdioecious species, both in animals and in plants, appear to have evolved from a dioecious rather than a hermaphroditic ancestor (Pannell 2002; Weeks et al. 2006).

Transitions from dioecy to hermaphroditism by way of androdioecy have been studied in only two plant species in much detail. In the wind-pollinated North American perennial herb *Datisca glomerata*, hermaphrodites appear to be modified (pollen-producing) females with the same sex determination as females in its dioecious sister species *Datisca cannabina* (Wolf et al. 2001). In the European herb *Mercurialis annua* (fig. 1), which is also wind-pollinated, androdioecy

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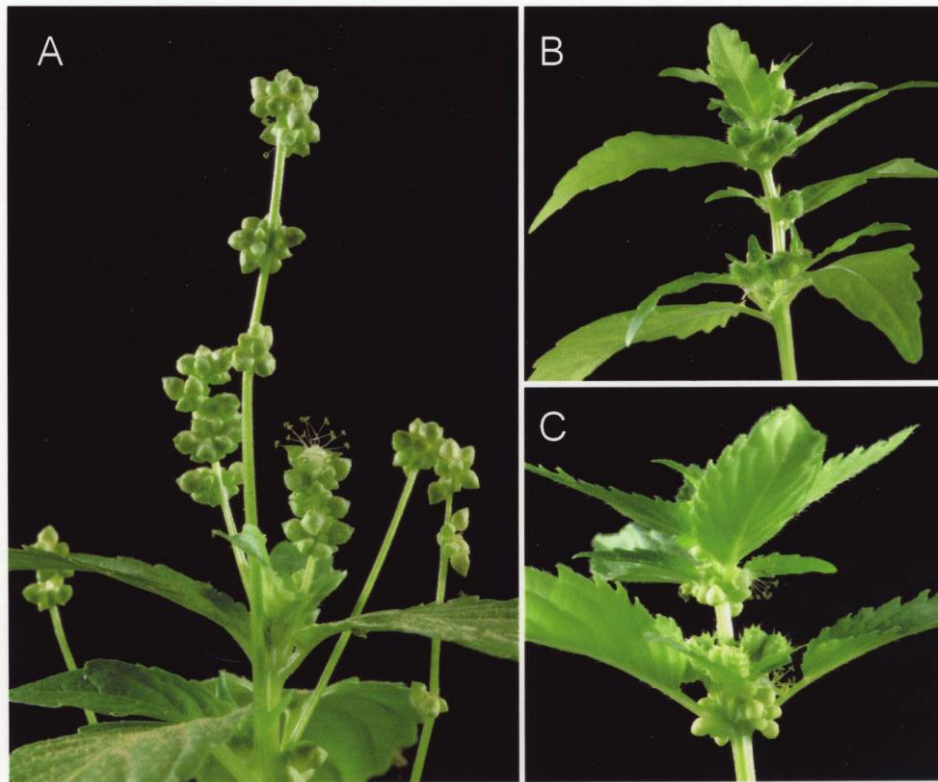


Fig. 1 Male (A), female (B), and monoecious (C) individuals of *Mercurialis annua*. The male flowers on the male and the monoecious individuals have the same morphology, but male flowers of male plants are held on erect peduncles. Also, the female flowers of both female and monoecious plants have the same morphology and placement in the leaf axils (in the photographs, female flowers have already set fruit). Males of diploid and hexaploid populations have the same morphology. Monoecious plants are effectively females that produce staminate flowers around their pistillate flowers.

is found only in polyploid populations of a complex in which dioecy is clearly the ancestral trait (Durand 1963; Durand and Durand 1992; Pannell 1997*d*; Pannell et al. 2004). *Mercurialis annua* is unusual in the extraordinary diversity of sexual systems it displays across its geographic range; this variation makes it a useful study system in which to address questions concerning evolutionary transitions between sexual systems and the origins and maintenance of androdioecy.

In this article, we present new data that advance our understanding of transitions in the sexual system of *M. annua* in two ways. First, we ask how closely the spatial transition between monoecy and androdioecy in a hitherto poorly studied part of the species' range on the Atlantic coast of Morocco corresponds to a shift between tetraploidy and hexaploidy. This question is important because elsewhere in the distributional range of the species complex, spatial (and perhaps evolutionary) transitions in the sexual system and ploidy levels are confounded; instances in which sexual-system transitions are uncoupled from those in ploidy provide particularly fertile ground for invoking the maintenance of different sexual systems by natural selection (rather than as a result of the historical distribution of ploidy levels). Second, we present results of an experiment that asks how the mating system is affected by

plastic responses to resource availability in the relative allocation to male and female functions. We address both these issues within the context of a synthesis of recent work on *M. annua*. We begin by considering the phylogenetic and phylogeographic history of the species complex in Europe. We then review empirical tests of a hypothesis that invokes differential extinction-colonization dynamics in a metapopulation to explain the maintenance of different sexual systems. Finally, we consider the potential importance of phenotypic plasticity in sex allocation in regulating the selection of combined versus separate sexes. Overall, we highlight the importance of both shifts in the genetic system associated with polyploidization and hybridization and shifts in the ecological and demographic context of selection for transitions between sexual systems.

Transitions in the Sexual System: The Role of Polyploidy and Hybridization

Mercurialis annua belongs to a small European genus in the Euphorbiaceae that comprises mainly dioecious woody or herbaceous perennials. Phylogenetic reconstruction of the

genus by Krahenbuhl et al. (2002), based on ITS sequence analysis, indicated that dioecy and perenniality are ancestral in the genus and that monoecy has evolved on at least two independent occasions, one of which was in the annual polyploid complex *M. annua*. This complex comprises ploidy levels ranging from diploid through at least 12-ploid, with dioecy confined to the diploids and monoecy found only in the polyploid lineages. Here, the tetraploids, octaploids, and higher ploidy levels are exclusively monoecious, but the hexaploids show remarkable variation in their sexual systems, with populations ranging from monoecy through androdioecy to subdioecy in various parts of their range (Durand 1963; Durand and Durand 1992).

Early studies of morphology and meiotic pairing behavior suggested that *M. annua* was an autopolyploid series and that polyploidization had precipitated the evolution of monoecy (Durand 1963). However, more recent analysis identified at least two divergent ITS paralogues in hexaploid populations of *M. annua*, only one of which occurred in diploid and tetraploid individuals (Obbard et al. 2006b). The other ITS sequence, absent in diploids and tetraploids, was found in *Mercurialis huetii*, a diploid sister species to *M. annua*, which has the same number of chromosomes and is also annual and dioecious. Obbard et al. (2006b) interpreted these results as evidence for hybridization between tetraploid *M. annua* and *M. huetii*, yielding triploids and followed by polyploidization to produce the hexaploid lineage (fig. 2). This hypothesis requires further testing with more exhaustive sampling of genotypes and loci, but preliminary results from microsatellite loci, some of which amplify only in hexaploid *M. annua* and *M. huetii*, are consistent with the ITS sequence analysis (P. Rymer, H. Stone, G. Korbecka, and J. R. Pannell, unpublished data).

Polyploidy represents a dramatic shift in the genetic system of a lineage. Not only may it alter the expression of inbreeding depression, potentially allowing the spread of self-fertilization in a population (Ronfort 1999; Husband et al. 2008), but it may also alter a broad range of phenotypic traits that may allow a lineage to occupy new and different habitats; i.e., it can cause ecological shifts that may also favor a transition in the sexual system of a population (Stebbins 1950; Pannell et al. 2004). Pannell et al. (2004) reviewed the interactions that are expected to occur between ploidy and sexual-system evolution. Their main conclusion was that the complexities involved make general predictions difficult. For example, on the one hand, polyploidization may allow self-fertile hermaphroditism to replace dioecy by causing a reduction in inbreeding depression (Lande and Schemske 1985; Pannell et al. 2004; Husband et al. 2008). On the other hand, genome duplication can cause the breakdown of self-incompatibility in hermaphroditic populations, allowing dioecy to evolve as an alternative outcrossing mechanism (Miller and Venable 2000; but see Mable 2004; Pannell et al. 2004). Thus, it can be tempting to invoke polyploidization as a cause of transitions both from hermaphroditism to dioecy and vice versa. In the case of *M. annua*, it would seem not only that polyploidization initiated the shift from dioecy to hermaphroditism but also that it has not precluded the maintenance of males in hexaploid populations of *M. annua* or the existence of dioecy in the newly discovered tetraploid species *Mercurialis canariensis* (Obbard et al. 2005).

Phylogeography and Regional Transitions in the Sexual System

Spatial transitions in the sexual system of *Mercurialis annua* in Europe (fig. 3) correspond, to an important extent (though not exclusively; see below), with transitions in the ploidy level. Fully dioecious populations are exclusively diploid; these are widespread throughout Europe. In contrast, populations containing hermaphrodites (with or without males) are polyploids; these are largely restricted to the western Mediterranean Basin and northwestern Africa. Tetraploids occur south of Rabat on the Atlantic coast of Morocco, and these meet with hexaploid populations to the north. Tetraploids are hermaphroditic, whereas the hexaploids, which are very widespread in northwestern Morocco and around the coast of the Iberian Peninsula, are variously hermaphroditic or androdioecious. Hexaploid populations meet the diploids at two contact zones in northeastern and northwestern Spain (Durand 1963; Obbard et al. 2006b).

Patterns of allelic richness and genetic diversity at several isozyme loci suggest that the Spanish diploid-hexaploid transitions are secondary contact zones, with diploids having expanded across Europe from an eastern Mediterranean refugium and the hexaploids having moved north along the coasts of the Iberian Peninsula from southern Spain or North Africa (Obbard et al. 2006b). This expansion of the geographic range of diploid *M. annua* appears to be continuing apace, with surveys suggesting that the diploids have displaced the hexaploids by some 80 and 200 km within about four decades in northeastern and northwestern Spain, respectively, apparently both as a result of diploid superiority in their growth (Buggs and Pannell 2007) and through the ability of diploids to swamp the monoecious hexaploids with the large amounts of pollen that diploid males produce (Buggs and Pannell 2006).

The higher pollen production of diploid males over hermaphrodites provides a plausible explanation for the rapid displacement of hermaphroditic populations by the dioecious lineage. However, what should we predict for a contact zone between dioecy and androdioecy, where males co-occur with the hermaphrodites? This is a pertinent question because, should the dioecious lineage continue its advance down the coasts of the Iberian Peninsula, it will soon encounter androdioecious populations farther south. Dorken and Pannell (2007) addressed this question, using spatially explicit computer simulations. They expected that the presence of males in androdioecious hexaploid populations might slow the diploid advance by competing with the diploid males to sire outcrossed progeny. However, because range expansion requires recurrent successful colonization (Baker 1955; Pannell and Barrett 1998), the occurrence of males with monoecious individuals actually diluted one of the benefits that monoecy has over dioecy: the advantage of reproductive assurance during colonization. Dorken and Pannell's (2007) simulations therefore predict that the diploid advance might accelerate when androdioecious regions are encountered, and they recall the complexities that can result when selection acts on plant reproductive strategies at both the population and metapopulation levels (Barrett and Pannell 1999; also see next section).

The transitions between dioecy and hermaphroditism in northern Spain, described above, are fully confounded by

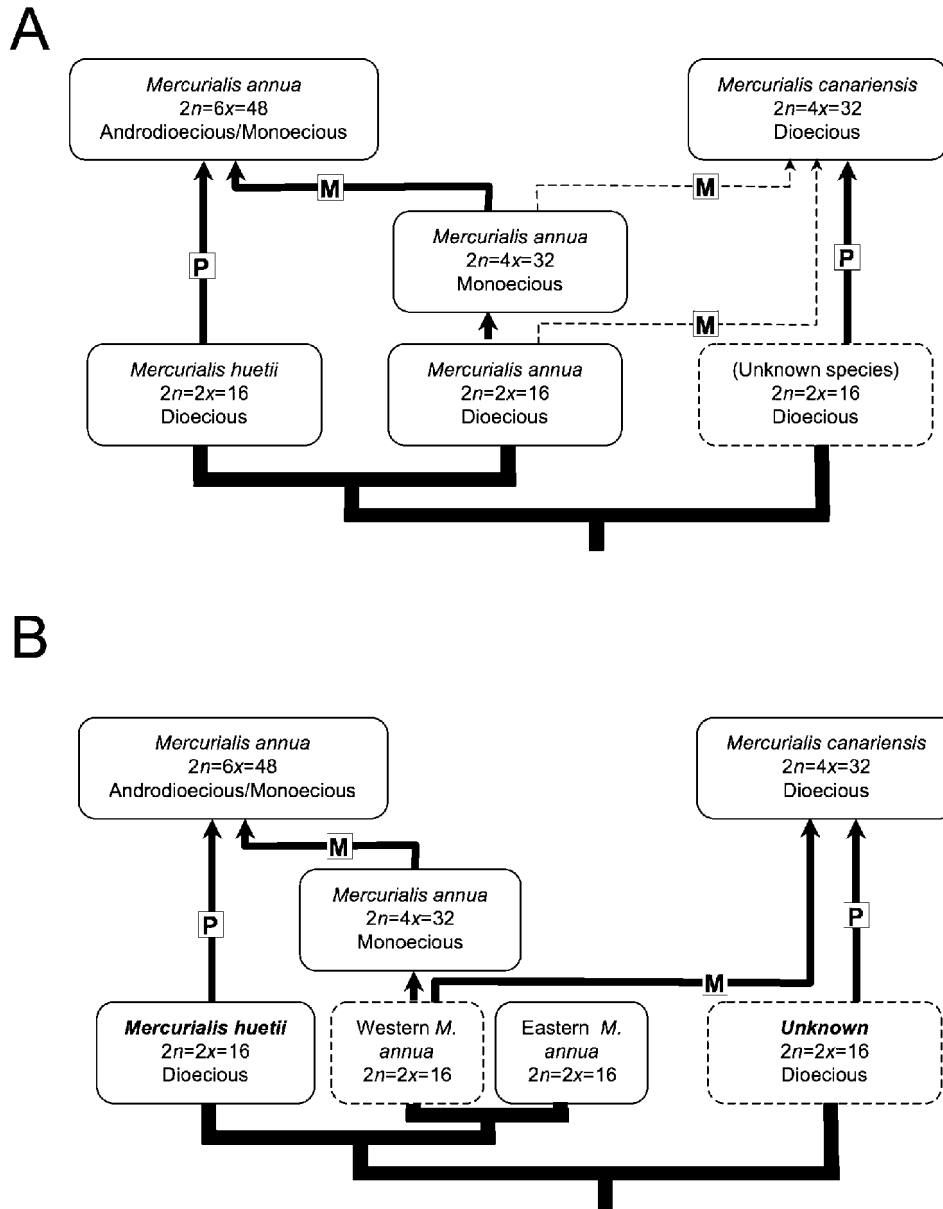


Fig. 2 Hypotheses for the relationships between the annual lineages of *Mercurialis*. Heavy lines indicate the phylogenetic relationships between diploid species, and thin arrows show polyploidization or hybridization events; *M* indicates proposed maternal parentage, and *P* indicates proposed paternal parentage. *A*, Diploid *M. annua* proposed as the parent of polyploid *M. annua*. The heterogeneous ITS types present in hexaploid *M. annua* show that it has an allopolyploid origin through hybridization between *M. annua* and *M. huetii*, and the hexaploid chromosome complement is consistent with hybridization between a tetraploid and a diploid lineage, followed by chromosome doubling. ITS data also show *M. canariensis* to be allopolyploid in origin, probably a hybrid between *M. annua* s.l. and an unknown taxon (dashed box). Chloroplast sequence similarity to *M. annua* s.l. suggests that *M. annua* s.l. was the maternal parent, and chromosome numbers are consistent with both parents being diploid. *B*, Given the greater similarity between ITS sequences from *M. canariensis* and tetraploid and hexaploid *M. annua*, the diploid progenitor of these taxa may have been an earlier western lineage of *M. annua*, divergent from the lineage that has recently expanded from the east. From Obbard et al. (2006a).

shifts between diploidy and hexaploidy. However, transitions also occur between androdioecy and hermaphroditism elsewhere in the Iberian Peninsula and in Morocco. In the Iberian Peninsula, these transitions occur within the hexaploid lineage, but in Morocco, the transition occurs along the Atlantic coast in a region broadly coincident with a transition between hexaploidy in the north and tetraploidy in the south

(Durand 1963). To determine whether these two transitions coincide precisely, we used flow cytometry to assay the ploidy level of 22 populations at intervals along the Atlantic coast of Morocco (fig. 4). For the flow cytometry measurements, we used leaf material from seeds grown in the greenhouse. Material was prepared using the “LB01” method of Dolezel et al. (1989) and *Lycopersicon esculentum* cv. Gardener’s Delight

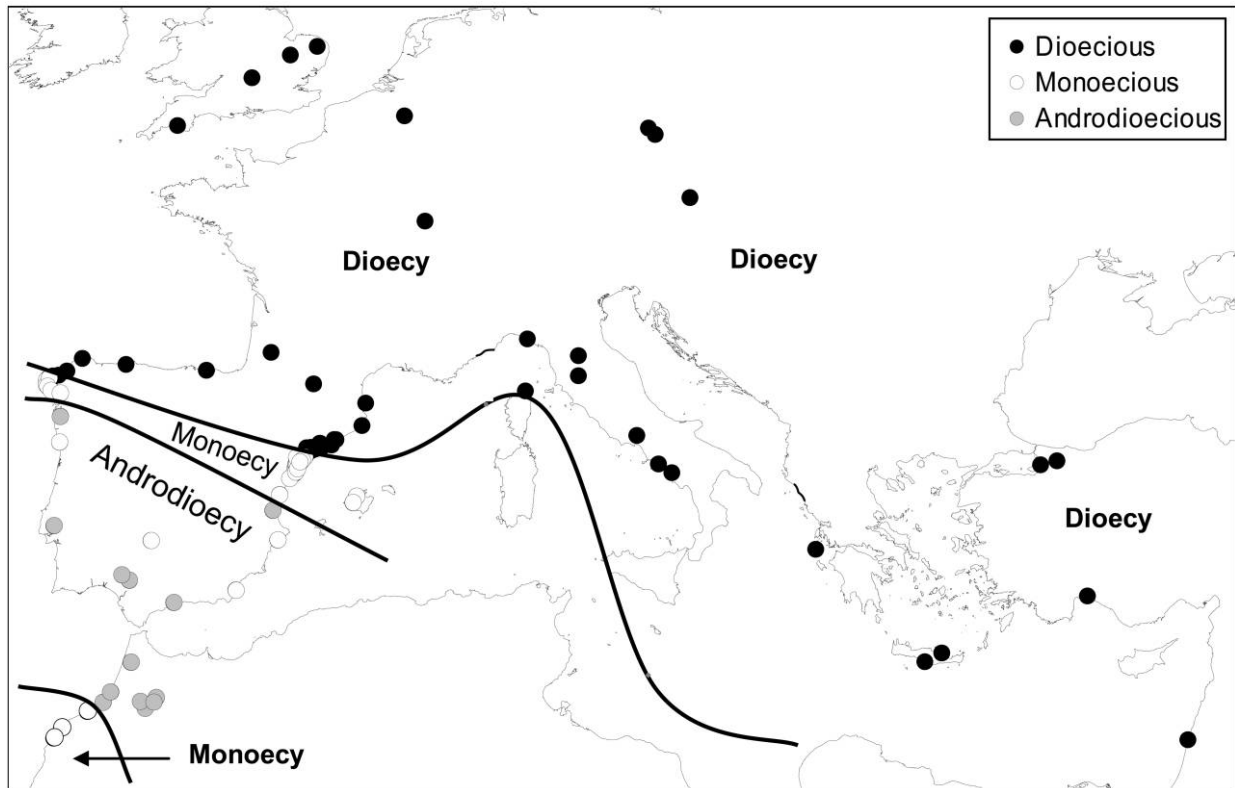


Fig. 3 Distribution of *Mercurialis annua* in Europe and around the Mediterranean Basin. In the north and east of this range, *M. annua* is dioecious and diploid, whereas in Iberia and North Africa, it is monoecious (and androdioecious) and polyploid. Circles indicate the locations of seed collections used to estimate patterns of diversity by Obbard et al. (2006b). Regions marked “Dioecy,” “Monoecy,” and “Androdioecy” denote zones occupied by the corresponding sexual systems (see “Phylogeography and Regional Transitions in the Sexual System”). Modified from Obbard et al. (2006b).

leaf material as a standard. Each sample was assessed on a Becton Dickinson FACScan flow cytometer and analyzed using Becton Dickinson CellQuest software (BD BioSciences, Franklin Lakes, NJ).

Our results indicate that the transition between tetraploidy and hexaploidy in Morocco occurs some 100 km south of the monoecy-androdioecy transition, which is located at Rabat; males are found only in populations north of this point (fig. 4). (Note that two tetraploid populations were found north of the major ploidy transition within the hexaploid zone. Such populations are likely to be the result of earth-moving roadworks, although they might also be remnant populations following a hexaploid advance south; see Buggs and Pannell 2006.) The geographic shift in the sexual system is thus uncoupled from the shift in ploidy in Morocco, and it occurs within the hexaploid lineage just as it does in the other monoecy-androdioecy transitions in the Iberian Peninsula. This poses the question of why the sexual system should change over geographical space, given that populations on both sides of these transitions have the same genetic system and appear to exchange genes (Obbard et al. 2006b).

Mechanisms of Transitions among Sexual Systems: The Role of Metapopulation Dynamics

To explain the maintenance of hermaphroditism versus dioecy or androdioecy in different regions of the range of hexa-

loid *Mercurialis annua*, Pannell (1997c) hypothesized a metapopulation model that invokes differential selection for reproductive assurance during colonization. Because only self-fertile hermaphrodites, and not males, can colonize unoccupied habitat on their own, we should expect extinction-colonization dynamics to reduce the frequency of males at the metapopulation (i.e., regional) level. At the same time, hermaphrodites with female-biased sex allocation should be favored by selection during colonization over those with more equal sex allocation because their populations will grow more quickly (note that selection for female-biased sex allocation at the metapopulation level can be seen equivalently in terms of local mate competition during selfing or in terms of deme-level selection; see Frank 1986). As populations grow denser after colonization, the selective advantage of maleness increases, both because of the availability of large numbers of ovules to fertilize and because of the low numbers of pollen grains produced by hermaphrodites with which they must compete. Thus, whereas males are disfavored during colonization, they may be favored as migrants (or, probably much more rarely, as new mutants) into established demes. Under this model, whether males are found at a regional level depends largely on the balance between these two opposing selective forces (Pannell 1997a; see also Pannell 2001).

The metapopulation model makes a number of predictions that have recently been tested. One demographic prediction is that, if the regional absence of males is the result of rapid

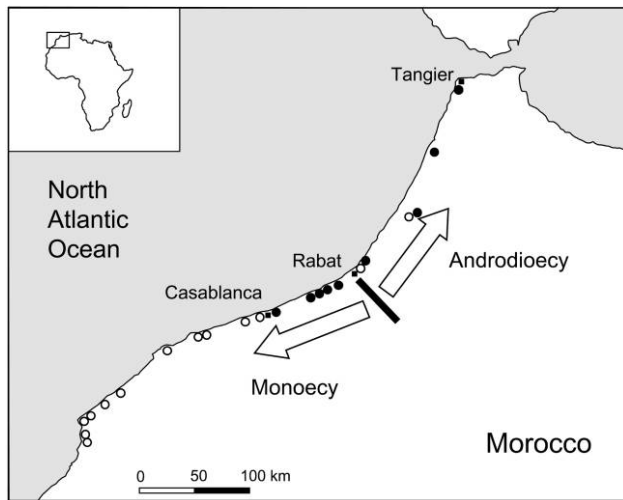


Fig. 4 Map showing the locations of 22 populations of *Mercurialis annua* for which the ploidy level was determined along the Atlantic coast of Morocco. Open and filled circles represent tetraploid and hexaploid populations, respectively. The solid line near Rabat indicates the point of transition between monoecious populations (to the south) and androdioecious populations (to the north). Two tetraploid populations were found within the area occupied principally by hexaploid populations.

population turnover compared with regions in which males occur, then populations in monoecious regions ought to be smaller than those in androdioecious regions (because they are, on average, younger). Another prediction is that rates of habitat occupancy should be lower in monoecious than in androdioecious regions if extinction-colonization rates are higher in the former (Gaston et al. 2000; Freckleton et al. 2005). Patterns of occupancy and abundance observed across several clines in the sexual system of *M. annua* in Spain were consistent with both these predictions (Eppley and Pannell 2007b).

From a population genetics perspective, the metapopulation model predicts that monoecious populations should have lower genetic variation and should be more strongly differentiated from one another than androdioecious populations. This is because monoecious populations are expected to display genetic signatures of more recent colonization bottlenecks that subsequent migration has not had time to erase (e.g., Slatkin 1977; Wade and McCauley 1988; Pannell and Charlesworth 2000). To test these predictions, Obbard et al. (2006b) measured genetic diversity in hexaploid monoecious and androdioecious populations across their range in the Iberian Peninsula and North Africa and in dioecious populations across their European range. They found that, even after the effects of the hypothesized range expansion had been accounted for (see “Phylogeography and Regional Transitions in the Sexual System”), hexaploid androdioecious populations were strikingly more diverse than their monoecious counterparts, as expected. Moreover, whereas pairwise genetic differentiation among hexaploid monoecious populations was highly variable and often large, pairs of androdioecious populations were invariably very similar, as were dioecious

populations. These patterns suggest a history of homogenizing gene flow among androdioecious populations and among dioecious populations (though not between androdioecious and dioecious populations, owing to their different ploidy levels; Obbard et al. 2006b). Although populations of *M. annua* with separate sexes might be linked by more gene flow because they contain males that disperse more pollen than hermaphrodites, such an explanation would not account for the low diversity of monoecious populations occurring within androdioecious regions (Obbard et al. 2006b).

A key assumption of the metapopulation model is that selfing rates are density dependent: hermaphrodites self-fertilize their progeny when mates are absent, but opportunities for outcrossing increase when populations grow. Eppley and Pannell (2007a) tested this assumption by estimating selfing rates in populations growing at different densities. Selfing rates were high when individuals were more than ca. 30 cm apart but quickly dropped in denser stands, as predicted by the model. Eppley and Pannell (2007a) used their results to predict the threshold density below which males should be excluded from a population and above which their maintenance was assured. This threshold density was much lower than commonly measured in purely monoecious populations, indicating that the presence of males in a population is indeed migration limited (Eppley and Pannell 2007a).

Evolution of Androdioecy: Sex Allocation and Inflorescence Structure

We have seen that dense populations of *Mercurialis annua* are largely outcrossing, easing the potential invasion of males into hermaphroditic populations. But how do males surpass the twofold threshold in pollen production required for their maintenance at frequencies greater than zero (Lloyd 1975; Charlesworth and Charlesworth 1978; Charlesworth 1984)? The metapopulation hypothesis suggests one mechanism by invoking selection for female-biased sex allocation in the hermaphrodites. Thus, if hermaphrodites allocate a proportion $x < 0.5$ of their reproductive resources to their male function, then female-sterile individuals that divert all their resources otherwise invested in seeds to pollen production will be able to disperse $1/x > 2$ units of pollen relative to the pollen produced by hermaphrodites. Full “compensation” in resource allocation between male and female functions would therefore be sufficient for male invasion as long as the hermaphrodites are female biased in their sex allocation.

Another way in which males might cross the twofold invasion threshold is by dispersing the pollen they produce better than hermaphrodites. Assume again that hermaphrodites allocate a proportion x of their resources to pollen production and that males allocate a proportion 1.0. Assume further that pollen grains dispersed by males are γ times more likely to find a receptive stigma than those dispersed by hermaphrodites. Then males should enjoy a relative siring success of γ/x . Clearly, if $\gamma > 1.0$, then males might invade a population of hermaphrodites even if $x = 0.5$, i.e., the population is unbiased in its sex allocation. Because we expect the sex allocation of outcrossing hermaphrodites to be ca. 0.5, if the male and female fitness gain curves are not very different

(Lloyd and Bawa 1984), this reasoning shows that the condition $\gamma > 1.0$ ought to be sufficient for the evolution of maleness fairly generally. Eppley and Pannell (2007a) tested this idea and estimated a value of $\gamma = 1.6$.

That males are so much better than hermaphrodites at dispersing their pollen is almost certainly due to the differences between the two morphs in their inflorescence architectures. Although monoecious plants have a female morphology, with male flowers held around a sessile female flower in the leaf axils, males disperse their pollen from flowers held above the plant on long, erect peduncles. Inflorescences such as those of *M. annua* males are widely found in wind-pollinated herbs and are expected to increase the siring success of pollen by allowing it to travel farther after release (Levin and Kerster 1974; Niklas 1985; Burd and Allen 1988). Such adaptations almost certainly give rise to an accelerating male fitness gain curve, and they should thus stabilize the maintenance of separate sexes (Charnov et al. 1976). It is poorly understood whether the evolution of such inflorescences is more likely to precede, and thus to precipitate, a transition from hermaphroditism to dioecy or to follow the evolution of dioecy and wind pollination. However, transitions between wind pollination and dioecy often go hand-in-hand (Renner and Ricklefs 1995; Wallander 2001; Friedman and Barrett 2008), and they may coincide with secondary sexual adaptations such as those found in *M. annua* (see Weller et al. 1998, 2006; Karrenberg et al. 2002; Friedman and Harder 2004; Golonka et al. 2005).

Maintenance and Breakdown of Androdioecy: The Role of Phenotypic Plasticity

Sex expression is notoriously labile in plants, with males and, less often, females of dioecious populations frequently producing flowers of the opposite sex (Lloyd and Bawa 1984; Korpelainen 1998). In gynodioecious and androdioecious species, the hermaphrodites too may vary in their sex allocation in response to environmental cues (reviewed by Delph and Wolf [2005]). Whereas the implications and functional significance of complete gender switches remain poorly understood, more subtle expressions of sexual lability may have important consequences for sexual-system evolution, e.g., by conferring upon a lineage an ability to self-fertilize after long-distance dispersal (Baker and Cox 1984). Plasticity in the sex allocation of hermaphrodites is probably driven by variation in the marginal cost of resources needed for reproduction or in changes to the shapes of fitness gain curves (Lloyd and Bawa 1984; Klinkhamer et al. 1997), and it has important implications for sexual-system evolution through its effect on the maintenance of males or females.

The phenotypic plasticity in sex allocation of *Mercurialis annua* hermaphrodites should have important implications for the frequency of males that can be maintained in androdioecious populations. Indeed, along with metapopulation dynamics, it might be a further factor underlying the large among-population variation observed in male frequencies of the species. As argued by Delph (2003), plasticity in sex allocation by hermaphrodites can change the relative fertility of

unisexual and hermaphrodite plants (see also Delph and Lloyd 1991). Because the relative pollen or seed fertility of unisexuals and hermaphrodites regulates their equilibrium frequencies (Lloyd 1976), such plasticity can cause variation in unisexual frequencies across ecological gradients (Delph 1990; Delph and Lloyd 1991; Asikainen and Mutikainen 2003; Barr 2004). For example, males always allocate all of their reproductive resources to pollen production (i.e., $x_m = 1$). If hermaphrodites change the proportion of resources allocated to pollen in response to an ecological gradient (i.e., x_h varies across the gradient), then this also changes the relative pollen fertilities of males and hermaphrodites (i.e., $r = x_m/x_h$). Half of the progeny sired by males are, on average, male, via the segregation of dominant male-determining alleles (Pannell 1997b). Thus, differences in r across environmental gradients will affect the relative frequency of male-determining pollen in the population and the frequency of males at equilibrium. Interestingly, such a scenario would concur with Darwin's (1877) reasoning that unfavorable environmental conditions can lead to an increased separation of the sexes and follows similar patterns shown by other species (reviewed by Delph and Wolf [2005]).

In a previous experiment, M. E. Dorken and J. R. Pannell (unpublished manuscript) found that plant density governs evolutionary trajectories in male frequencies by affecting the magnitude of r . Under high plant densities, r was more than three times higher than under low densities (and see Pannell 1997c). In the next generation, male frequencies were 38% higher among the progeny of plants grown under high densities (M. E. Dorken and J. R. Pannell, unpublished manuscript). Density can affect male siring success in two ways. First, density may affect the local availability of resources by increasing competition among plants. Second, because pollen is dispersed locally (Eppley and Pannell 2007a), density directly affects male siring ability. We attempted to dissect these confounded effects by controlling plant density and manipulating resource availability. Does resource availability affect the magnitude of r ? If so, does variation in resource availability regulate male frequencies in *M. annua*?

To address these questions, we mixed seeds from five nearby sites in Morocco, each with high frequencies of males (i.e., between 25% and 47%). We grew plants from seed in 12 3 × 3-m raised beds in standardized, low-nutrient soil mixtures at the Wytham Field Lab between July and October 2004. We added slow-release nutrient fertilizer pellets (Osmocote, Scotts, Marysville, OH) at the recommended dosage to half of the beds, using a randomized block design with three blocks. On average, there were 566 ± 97 (SD) plants in the high-resource plots and 566 ± 42 (SD) plants in the low-resource plots. We maintained high male frequencies in each plot by transplanting males from additional similar plots not included in this analysis here (average male frequency in high-resource plots = 52.8% ± 1.4% [SE], in low-resource plots = 52.5% ± 8.2% [SE]). At the end of the experiment, we harvested a standardized sample of 20 hermaphrodite and 10 male plants from each plot by sampling individuals at regular intervals along four evenly spaced transects. Following Pannell (1997c), we calculated hermaphrodite allocation to male function (r) as the aboveground proportion of biomass allocated to pollen of hermaphrodites (π_h) relative to

that of males (π_m ; i.e., $r = \pi_m/\pi_h$). Thus, lower values of r indicate higher hermaphrodite allocation to pollen.

We found that hermaphrodites grown under high-resource conditions (average $r = 11.2 \pm 0.9$ [SE]) had nearly double the allocation to pollen of plants grown under low-resource conditions (average $r = 21.6 \pm 2.3$ [SE]; fig. 5a; two-tailed t -test with equal variances: $t = -4.28$, $df = 10$, $P < 0.005$). At the end of September, we harvested all hermaphrodites in

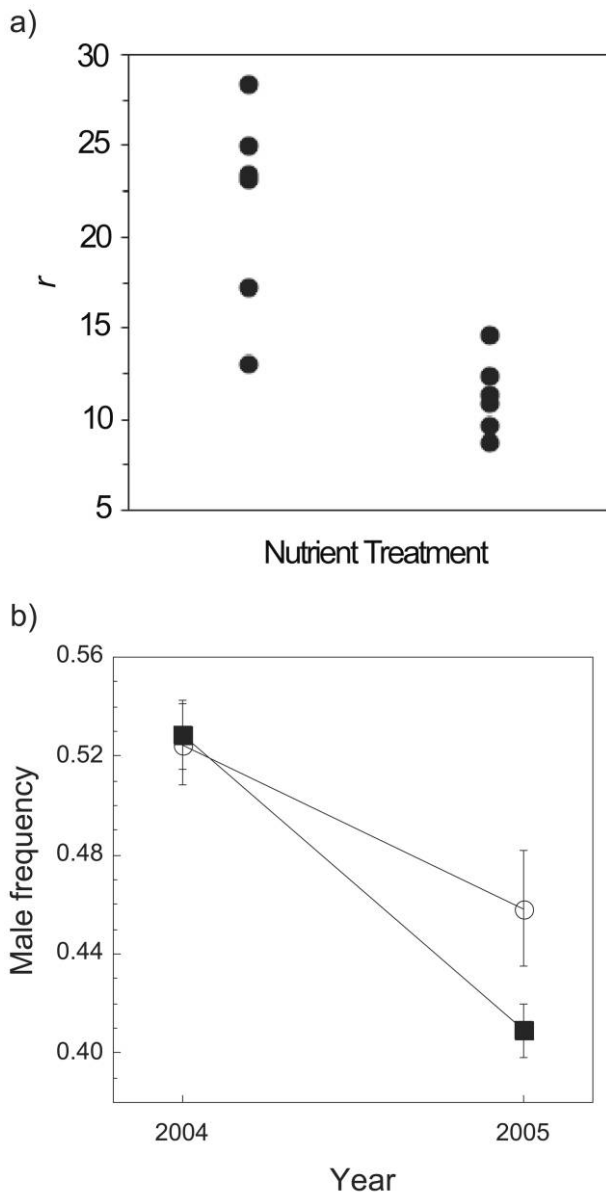


Fig. 5 Phenotypic plasticity in hermaphrodite sex allocation affects evolutionary trajectories in male frequencies. *a*, The relative pollen production (r) of males, compared with that of hermaphrodites, differs between low- and high-resource conditions. *b*, Because the pollen fertility of males relative to that of hermaphrodites regulates the frequency of males, different values of r between low- (circles) and high-resource conditions (squares) lead to divergence in male frequencies in the next generation. In *b*, error bars represent standard errors.

a plot and collected their seed in bulk, and we sowed an average of 1500 seeds (estimated by weighing out standard amounts of seeds) back into the plot in June 2005. Because hermaphrodites had higher allocation to pollen in high-resource conditions, the proportion of progeny sired by males should have been lower than that under low-resource conditions. Moreover, because maleness is governed by the segregation of a dominant Mendelian allele (Pannell 1997b), we predicted that the frequency of males among the progeny of plants grown under high-resource conditions should be lower than that among those from low-resource conditions. Our results are consistent with this prediction (fig. 5). The average frequency of males from high-resource plots was $40.9\% \pm 1.1\%$ (SE; average number of plants per plot = 720 ± 74 [SE]), compared with $45.8\% \pm 1.6\%$ (SE) for low-resource plots (average number of plants per plot = 501 ± 83 [SE]; one-tailed t -test with equal variances: $t = -1.92$, $df = 10$, $P < 0.05$).

Our results indicate that plasticity in sex allocation regulates the frequency of unisexual plants in gender-dimorphic populations, supporting the prediction made by Delph (2003). Even though our experiment was conducted across a single generation, we found considerable changes in male frequencies in response to environmental conditions. Specifically, we found that male frequencies not only can respond to density, which regulates male siring ability (as predicted by Eppley and Pannell 2007a), but also can respond directly to variation in r imposed by variation in the resource status of hermaphrodites. These results demonstrate the importance of phenotypic plasticity in hermaphrodite sex allocation for regulating male frequencies in androdioecious *M. annua*.

Genetics of Sex Determination and Sex Allocation in *Mercurialis annua*

The genetic basis of sex expression in dioecious *Mercurialis annua* was studied intensively by Durand and co-workers (Louis and Durand 1978; Dauphin-Guerin et al. 1980; Durand and Durand 1991a, 1991b). On the basis of crossing experiments using a small sample of original genotypes, they concluded that sex was determined by epistatic interactions between alleles segregating at three independently segregating loci. The fact that family sex ratios are often strongly male or female biased, despite a 1 : 1 sex ratio at the population level (J. R. Pannell, personal observations), would seem to confirm that more than one locus is involved, but results of recent genetic analysis based on sequence-characterized amplified-region (SCAR) markers were consistent with a single locus (Khadka et al. 2002). Pannell (1997b) concluded that maleness in an androdioecious population was determined by the presence of a single dominant allele, but his analysis also pointed to the importance of a plastic component of sex expression. Clearly, much remains to be learned about sex determination in *M. annua* and about the evolution of sex determination in the complex through its sexual-system transitions.

It is clear from the studies reviewed above that sex allocation in hermaphrodites of *M. annua* is also highly variable, albeit as a continuous trait. Much of this variation is evidently due to phenotypic plasticity, but comparisons between

Moroccan and Spanish populations suggest an important genetic component, too (J. R. Pannell, R. Berjano, and S. M. Eppley, unpublished manuscript); however, the architecture of this genetic variation in *M. annua* is unknown. If a more complete picture of past evolutionary transitions between sexual systems in the species complex is to emerge, we need further information about the quantitative genetics of sex allocation.

Two specific questions are currently the focus of attention of our research, and both relate to the so-called breeder's equation, $R = h^2S$, which relates the potential response of a population to selection, R , to the product of the selection coefficient, S , and the narrow-sense heritability of the trait, h^2 . Because $h^2 = V_A/V_P$, i.e., the fraction of total phenotypic variance that is due to additive genetic variance, the potential for response to selection on sex allocation will depend on both the absolute amount of additive genetic variance, V_A , and the size of V_P , which will be inflated by phenotypic plasticity (Falconer and Mackay 1996). The first question thus concerns how phenotypic plasticity in sex allocation in *M. annua* will affect responses to selection on pollen production. This question is of general importance for our understanding of transitions from gynodioecy and androdioecy to dioecy (Delph and Wolf 2005).

The second question concerns the size of V_A : how much additive genetic variance in sex allocation is present in populations of *M. annua* with different sexual systems, and how might this vary geographically? The geographic perspective on this question is important because range expansions, which are known to have occurred in *M. annua* (see "Phylogeography and Regional Transitions in the Sexual System"), typically involve repeated population bottlenecks that are expected to reduce quantitative genetic variation (Lande 1992). Indeed, range expansion in *M. annua* was inferred on the basis of reduced genetic variation at isozyme loci with distance from putative refugia (Obbard et al. 2006b). What we do not know is whether the hypothesized range expansions affected genetic variation in sex allocation, which is under frequency-dependent selection, differently. Current work is addressing this question through an assessment of the response to selection on pollen production in hermaphrodites sampled from populations at different latitudes in Spain and North Africa.

Conclusions

Mercurialis annua displays unusual variation in its sex expression at several levels in the genealogical hierarchy, including within and among genotypes, among populations in putative metapopulations, among regions in different parts of Europe, and between related species in a clade represented by lineages with reticulate phylogenetic relationships. This variation reflects a complex history of divergence, migration, and gene flow, which have all contributed to transitions between sexual systems. Research on *M. annua* has thrown light onto a number of themes in sexual-system evolution. These include the importance of ploidy, hybridization, and range expansions in regulating sexual-system variation and thus setting the stage on which natural selection then acts; the importance of phenotypic plasticity underlying much of the variation observed between individuals and its responsibility in regulating details of sex ratio evolution; the role of context-dependent mating in species characterized by demographic fluctuations and possible metapopulation dynamics; the adaptive value of sexual specialization for pollen dispersal under wind pollination; and the potential role of androdioecy in evolutionary paths between dioecy and hermaphroditism, a pathway about which very little was hitherto known. Future work aims to characterize the demographic processes hypothesized to have played a role in driving the observed sexual-system transitions at regional and local-patch scales as well as to describe the quantitative genetic architecture of sex determination and sex allocation across the phylogenetic and geographic range of the species complex.

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