VOL. 167, NO. 3 THE AMERICAN NATURALIST MARCH 2006

Sexual Systems and Population Genetic Structure in an Annual Plant: Testing the Metapopulation Model

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Submitted July 5, 2005; Accepted October 27, 2005; Electronically published February 14, 2006

ABSTRACT: The need for reproductive assurance during dispersal, along with the pressure of local mate competition, means that the importance of frequent or repeated colonization is implicit in the literature on sexual system evolution. However, there have been few empirical tests of the association between colonization history and sexual system in plants, and none within a single species. Here we use patterns of genetic diversity to provide such a test in the *Mercurialis annua* species complex, which spans the range of systems from self-compatible monoecy through androdioecy to dioecy. This variation has been hypothesized to result from differing patterns of metapopulation turnover and recolonization. Because monoecy should be favored during colonization, androdioecy and dioecy will be maintained only in regions with low rates of local extinction and recolonization, and these differences should also be reflected in patterns of neutral genetic diversity. We show that monoecious populations of *M. annua* display lower within-population genetic diversity than androdioecious populations and higher genetic differentiation than dioecious and androdioecious populations, as predicted by metapopulation models. In contrast, regional diversity in *M. annua* appears to be primarily a product of postglacial range expansion from two refugia in the eastern and western Mediterranean Basin.

Keywords: allopolyploid, androdioecy, *Mercurialis annua*, dioecy, genetic diversity, phylogeography.

Plants are strikingly diverse in their sexual systems (Barrett 2002). Much of this diversity can be understood in terms of the costs and benefits of inbreeding versus outbreeding, or in terms of trade-offs between investment in male versus female functions. The contrast between hermaphroditism and dioecy, or more generally between combined and separate sexes, is particularly interesting because it potentially highlights the outcomes of both of these trade-offs simultaneously (Charlesworth and Charlesworth 1981). Thus, while self-fertile hermaphrodites may be partially or wholly inbreeding and allocate resources to both sexual functions, males or females are obligate outcrossers and gender specialists. Theories for the evolution of combined versus separate sexes therefore naturally consider the implications of inbreeding versus outbreeding, the allocation of resources to the two sexual functions, or both (Charlesworth 1999).

Theoretical models that assume linear fitness gains with investment to both male and female functions predict that dioecy will be stable to the invasion of partially selfing hermaphrodites if inbreeding depression in selfed progeny is greater than one-half (Charlesworth and Charlesworth 1981). This condition is identical to that predicted for the invasion of selfing variants into an outcrossing hermaphroditic population and corresponds to the well-known automatic transmission advantage conferred upon selfing hermaphrodites (Fisher 1941). Apart from assuming linear fitness gains, models for the maintenance of dioecy versus hermaphroditism also make important implicit assumptions about pollination biology. In particular, they assume that seed set is not limited by the availability of mates or pollinators. Under pollen-limited seed set, dioecy is less stable to the invasion of self-fertile hermaphroditism because selfing confers the additional benefit of reproductive assurance (Maurice and Fleming 1995; Wolf and Takebayashi 2004). Thus, we expect an increased incidence of self-fertile hermaphroditism under conditions of low population or pollinator densities or in situations following long-distance dispersal and colonization by single individuals (Baker 1955, 1967).

The evolutionary stability of dioecy versus hermaphroditism is also predicted to depend on the shape of the relation between investment of resources to male and female functions and fitness (Charnov et al. 1976; Charnov

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Am. Nat. 2006. Vol. 167, pp. 354–366.

1982). Although inbreeding affects the shape of the gain curve (Charlesworth and Charlesworth 1981), a useful rule of thumb is that hermaphroditism will be selected when male or female gain curves (or both) reflect diminishing returns, so that increased investment yields ever smaller increases in fitness gained. Many characteristics of modular hermaphrodites with a sessile habit are thought to cause such saturating gain curves and might help to explain the high incidence of hermaphroditism in plants (Charnov et al. 1976). In contrast, if one or both of the fitness gain curves is accelerating, that is, if increased investment leads to ever-increasing gains in fitness, then dioecy is predicted to be stable to invasion of hermaphrodites (Charnov et al. 1976). The increased attractiveness of large floral displays to pollinators and large displays of fleshy fruits to seed dispersers are two traits that might cause accelerating fitness gain curves under certain circumstances. Similarly, accelerating fitness gain curves may result from morphological specialization by males or females (Eckhart 1999). For example, in wind-pollinated herbs, males tend to be taller than females and often possess inflorescences that differ markedly from those of females (Bickel and Freeman 1994; Eckhart 1999); such traits are likely to improve pollen dispersal (Klinkhamer et al. 1997).

The joint effects of inbreeding and the shape of the male and female fitness gain curves influence not only the stability of dioecy versus hermaphroditism but also the proportional allocation of resources to the two sexual functions in hermaphrodites. In large outcrossed populations in which the male and female gain curves are similar in shape, hermaphrodites should invest equally in the two sexual functions (Charnov 1982). However, in inbreeding populations, the sex allocation is expected to be female biased (Hamilton 1967). This is one of the best-tested predictions of life-history theory and has been shown to hold in a wide range of systems, from dioecious animals in which mating occurs between close relatives (West et al. 2000) to partially selfing hermaphroditic plants, which have low pollen : ovule ratios (Cruden 1977) and low investment toward the attraction and reward of pollinators. In hermaphrodites that frequently engage in long-distance dispersal, inbreeding rates are expected to be high, and sex allocation should similarly be female biased (Pannell 2001). This will apply particularly during the colonization phase, when all progeny may be self-fertilized. However, the population will remain effectively inbreeding even if selfing rates drop with population growth because local mating partners will still be closely related to one another if immigration rates are low (Frank 1986).

The importance of colonization for sexual system evolution is implicit in much of the literature dealing with the effects of inbreeding versus outbreeding and the trade-offs involved in the allocation of sex. However, there have been few good tests of these ideas. Ideally, one would seek a model system in which suitable variation in both the sexual system and colonization dynamics are displayed together, preferably within a single species. A great deal has been gained by adopting model species that vary in their sexual systems to test evolutionary hypotheses, but species that display both dioecy and hermaphroditism are rare (Costich 1995; Case and Barrett 2001; Dorken and Barrett 2004). Moreover, none of these species has been used to test hypotheses concerning the effect of colonization on the maintenance of combined versus separate sexes.

In this study, we conducted the first empirical test within a single species complex of the predicted association between the sexual system of a lineage and its history of colonization. We chose as our model the plant *Mercurialis annua* L. (Euphorbiaceae), a wind-pollinated weedy annual that occupies disturbed habitat over much of Europe (Durand 1963; Tutin et al. 1968). *Mercurialis annua* is particularly useful as a model in this context because of the remarkably broad variation it displays in its sex allocation in the Iberian Peninsula (Durand and Durand 1992). Moreover, not only are both dioecy and monoecy (functional hermaphroditism) widespread, but *M. annua* also displays the rare sexual system androdioecy, where males co-occur with self-compatible hermaphrodites (Pannell 1997*d*). Not only are these androdioecious populations intermediate in their sex allocation between dioecy and hermaphroditism (Pannell 1997*c*), but also the co-occurrence of individuals with either combined or separate sexes allows comparisons to be made between these allocation strategies within the same populations.

An ecological model involving colonization in the context of a metapopulation has been proposed as an explanation for much of the sexual system variation observed in *M. annua* (Pannell 2001; fig. 1). Essentially, this model recognizes dioecy as the evolutionarily stable strategy for the species in large, demographically stable populations, either as a result of the advantage of gender specialization (Pannell 1997*c*, 1997*d*) or as an outcrossing mechanism. In areas where colonization dynamics have been more important, the model proposes that self-fertile hermaphroditism has evolved as a result of selection for reproductive assurance during population establishment; these partially inbred populations should consequently also display female-biased sex allocation. Finally, androdioecy should occur in areas where the importance of colonization and population turnover is intermediate. Here, populations are more likely to be established by selfing hermaphrodites with female-biased sex allocation, but once populations have grown in size and selfing rates have fallen, immigrant males will be able to spread locally (Pan-

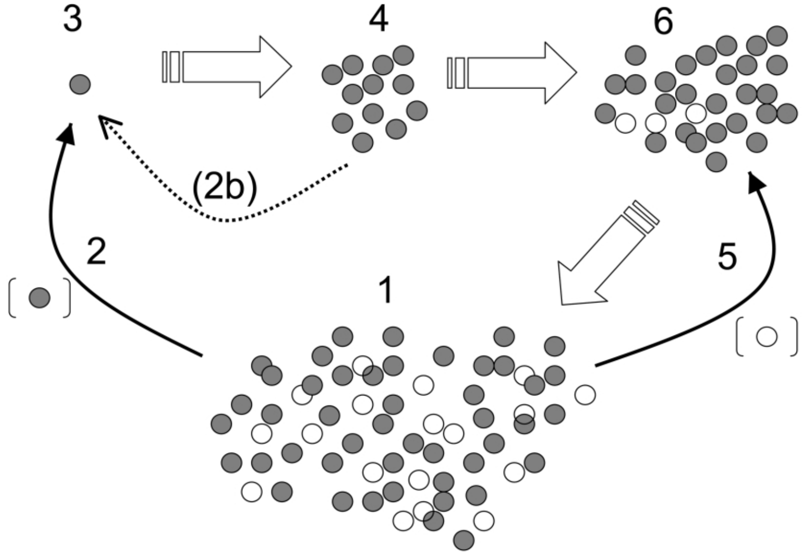

Figure 1: Hypothesized sexual system dynamics in hexaploid *Mercurialis annua.* Males and hermaphrodites are symbolized by open and filled circles, respectively; block arrows represent population growth; simple arrows represent dispersal. In the absence of significant population turnover, the evolutionarily stable strategy is thought to be dioecy. However, recurrent local extinction selects for monoecy, and over ecological timescales, this results in a patchwork of monoecious and androdioecious populations, depending on the rate of population turnover. Under these circumstances, established populations (1) disperse male and hermaphrodite propagules across the metapopulation (2 and 5, respectively). Only hermaphrodites can self-fertilize their ovules and thus establish populations as single individuals (2); in *M. annua*, these populations will lack males because hermaphrodites are homozygous for the hermaphrodite-determining allele (Pannell 1997*b*). New populations (3) are small and sparse and are initially highly selfing (4). As populations grow, outcrossing with neighbors becomes easier, and density-dependent selfing rates decline, allowing males to invade (5) and leading to an androdioecious population (6). A balance between extinction and recolonization maintains the age structure of the metapopulation. If population turnover is very high, populations do not persist long enough for males to immigrate; male invasion (5) thus does not occur. In this case, the largest populations are monoecious (4) rather than androdioecious (1, 5), so colonists come from monoecious populations (2b) rather than androdioecious populations (2); in other words, the cycle is "short-circuited," giving rise to a monoecious rather than an androdioecious metapopulation. (Adapted from Pannell et al. 2004.)

nell 1997*c*, 2001). In androdioecious regions, we therefore expect a mosaic of populations with different male frequencies to be maintained over ecological timescales, reflecting variation in their recent colonization and immigration histories (fig. 1).

The distribution of dioecy and androdioecy/monoecy, and the variation in male frequencies observed in androdioecious regions of *M. annua* are consistent with the metapopulation model (Durand 1963; Pannell 1997*c*, 1997*d*), but an independent test is needed (Pannell 1997*c*). Here, we conduct an indirect test of the model by comparing patterns of genetic variation within and among populations occurring in regions that differ in their sexual systems. If the rate of population turnover is greater than the rate of migration between established populations, within-population diversity is expected to be reduced, and genetic differentiation among populations is expected to be increased (reviewed in Pannell and Charlesworth 2000). Thus, we predicted lower within-population diversity and higher genetic differentiation among monoecious than among androdioecious or dioecious populations.

Metapopulation genetic theory also predicts a reduction in metapopulation-wide genetic diversity with increasing population turnover rates (Slatkin 1977; Whitlock and Barton 1997; Wakeley and Aliacar 2001; Pannell 2003). We would thus expect higher total diversity in dioecious and androdioecious regions than in monoecious regions. However, regional diversity levels are known to depend largely on more ancient genetic and demographic processes, such as past mutation rates and large-scale phylogeographic patterns of dispersal (Hewitt 2000; Charlesworth and Pannell 2001). Indeed, the signature of range expansion following Pleistocene glaciation has affected regional patterns of diversity in many European plants and animals (Hewitt 1999; Petit et al. 2003). Although information on weedy plant species is largely lacking, three patterns of postglacial range expansion have frequently been identified among animals and perennial plants in Europe, depending on the distribution of glacial refugia between Iberia, Italy, and the eastern Mediterranean Basin (Hewitt 1999; see "Discussion"). However, the locations of the Pleistocene refugia of *M. annua* are not yet known.

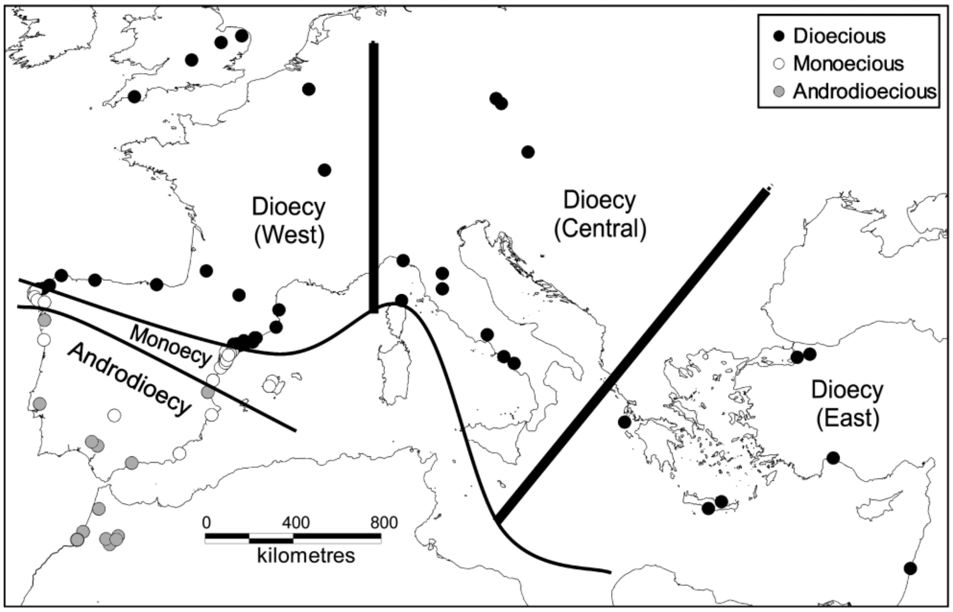

Figure 2: *Mercurialis annua* occurs all over northern Europe and around the Mediterranean Basin. In the north and east of this range it is dioecious and diploid, while in Iberia and north Africa it is monoecious (and androdioecious) and polyploid. Circles indicate the location of seed collections used in this study. Regions marked "dioecy," "monoecy," and "androdioecy" denote zones occupied by the corresponding sexual system. The "west," "central," and "east" subdivisions of the dioecious zone are arbitrary regions used to illustrate the effect of range expansion on genetic diversity.

It is possible that the dioecious and monoecious/androdioecious populations of *M. annua* in the Iberian Peninsula had different glacial refugia, so that the current distribution of sexual systems in the species might partly reflect different phylogeographic histories. For example, the distribution of dioecious and monoecious populations of the herb *Ecballium elaterium* in Spain has been attributed to range expansion from different refugia (Costich and Meagher 1992; see "Discussion"). Importantly, dioecious populations of *M. annua* are diploid ($2n = 16$), whereas the monoecious and androdioecious populations that we studied here are hexaploid (Durand 1963; Durand and Durand 1992). In order to identify potentially different refugia of the different sexual systems and ploidy levels of *M. annua*, and to reconstruct the history of range expansions before occupation of the Iberian Peninsula, we therefore sampled *M. annua* populations widely across Europe. Specifically, we analyzed the distribution of genetic diversity across Europe and northwest Africa to determine which of the three putative European refugia were most likely to have been occupied by the different lineages of *M. annua* during the Pleistocene. We thus interpret the current patterns of genetic diversity displayed by *M. annua* in the Iberian Peninsula in terms of both historical and ongoing demographic processes.

Methods

Sampling Regime

Seed was collected from approximately 20–40 (minimum 10, maximum ca. 100) seed-bearing individuals in each of 45 dioecious and 44 monoecious or androdioecious populations across the species' range in Europe (fig. 2; site locality information is available from the authors upon request). For sexual system comparisons, the geographical distribution range was divided into zones according to the predominant sexual system of the region. Comparisons were made between these zones, as well as between monoecious and androdioecious populations within the androdioecious zone, where they co-occur, and across all zones together. Androdioecious populations were conservatively defined as those with male frequencies >0.01. For the phylogeographic analysis, the dioecious zone was further divided into "west," "central," and "east" across Europe (fig. 2). At various times between November 2000 and August 2003, approximately 45 plants per population were established from seed in a glasshouse, with a mean of 48 individuals per dioecious population and 42 individuals per monoecious or androdioecious population.

Isozyme Extraction and Visualization

Six enzyme systems could be consistently resolved in diploid *Mercurialis annua*: AAT (aspartate aminotransferase E.C. 2.6.1.1), PGI (glucose-6-phosphate isomerase E.C. 5.3.1.9), PGM (two loci, phosphoglucomutase E.C. 5.4.2.2), ME (malic enzyme NADP$^+$ E.C. 1.1.1.40), 6-PGD (two loci, phosphogluconate dehydrogenase E.C. 1.1.1.44), and IDH (isocitrate dehydrogenase E.C. 1.1.1.42). Only five of these enzymes—all except PGM—could be reliably interpreted in hexaploid *M. annua*. Of these, ME was monomorphic in both diploids and polyploids.

Protocols and solutions were adapted from Weeden and Wendel (1990) and Wendel and Weeden (1990). For each individual, approximately 2 cm^2 of fresh young leaf tissue was ground with extraction buffer (1.21 g Tris-HCl, 0.04 g EDTA, 0.076 g KCl, 0.2g $MgCl_2 \cdot 6H_2O$, 4 g PVP, and 0.5 g PVPP in 100 mL stock, made up with 90 : 10 : 0.5 stock : DMSO : ß-mercaptoethanol) on a prechilled ceramic block. The resulting paste was soaked into Whatmann #3 paper wicks (3 mm × 8 mm) for loading into starch gels. Gels were made from 14% w/v hydrolyzed potato starch (StarchArt, Smithville, TX) and 0.25% w/v sucrose. Three buffer systems were used. Lithium borate gels (electrode buffer: 0.039 M LiOH, 0.263 M boric acid; gel buffer: 33 mM Tris base, 5 mM citric acid, 4 mM LiOH, 30 mM boric acid, pH 7.6) were stained for AAT, PGI, PGM, and ME. Morpholine citrate gels (electrode buffer: 0.04 M citric acid, 0.068 M N-[3-aminopropyl]-morpholine, pH 6.4; gel buffer: 1 : 14 dilution of the electrode buffer) were stained for 6-PGD. Tris citrate gels (electrode buffer: 0.135 M Tris base, 0.03 M citric acid, pH 8.3; gel buffer: 1 : 19 dilution of the electrode buffer) were stained for IDH. All gels were run at 4°C for approximately 6 h. Lithium borate, morpholine citrate, and Tris citrate gels were run at 300, 250, and 150 V, respectively. Gels were cut into slices (1 mm thick) and stained at 40°C. Staining solutions were adapted from Weeden and Wendel (1990) and Wendel and Weeden (1990). Reactions were stopped, and gels were stabilized after staining by removing the stain solution and adding 30 mL 25% v/v glycerol. Gels were scored from photographs taken at the time of staining.

Analysis of Isozyme Diversity

Diploid populations. We made full genetic interpretations for diploid isozyme banding patterns using published data on enzyme structure and compartmentalization in plants (Weeden and Wendel 1990; Wendel and Weeden 1990), in conjunction with artificial crosses where necessary (data not shown). Nei's gene diversity H_e (expected heterozygosity; Nei 1987), allelic richness corrected for sample size (El Mousadik and Petit 1996), and estimates of F_{IS} and F_{ST} (Weir 1984; Weir and Cockerham 1984) were calculated using FSTAT, version 2.9.3.2 (Goudet 1995). Genetic diversity and differentiation were calculated, first across all populations and then for each geographic zone depicted in figure 2. Significant differences between geographic regions were assessed using randomization tests as implemented in FSTAT.

Hexaploid populations. For polyploid individuals, gene duplication, multiple alleles, and the mode of inheritance can lead to practical and statistical complications in scoring genetic gels and interpreting summary statistics (e.g., Bever and Felber 1992; Ronfort et al. 1998). For polyploid individuals with disomic inheritance, the expectations for underlying genetic diversity and differentiation are the same as for diploid individuals under a given model of population structure. However, duplicate isozyme loci ("isoloci"; Waples 1988) often comigrate on the gel, and they have to be scored as from a single "locus," which prevents the application of standard diversity statistics that require genotype or allele frequencies for their calculation, such as Nei's (1987) gene diversity. Nevertheless, if the mode of inheritance is known, difficulties in scoring genetic data can largely be overcome through the use of approaches based on "allelic phenotypes" (e.g., Murdy and Carter 1985; Bayer and Crawford 1986; Rogers 2000; Berglund and Westerbergh 2001).

Hexaploid *M. annua* has disomic inheritance (Obbard 2004). Thus, allele dosage could not be reliably estimated from isozyme gels. Genetic diversity and differentiation statistics were therefore calculated using the program FDASH (D. J. Obbard, S. A. Harris, and J. R. Pannell, unpublished data). This program uses a measure of genetic diversity for use with allelic phenotypes, H', defined as the average number of alleles by which pairs of individuals differ. For example, a pair in which one individual carries alleles *abc* and the other carries alleles *bd* differs by three alleles, *a*, *c*, and *d*. This diversity measure can be calculated both within local populations (H'_S; here the average within-population diversity) and across many populations (H'_T). Together, these allow a measure of genetic differentiation, F'_{ST}, to be calculated as $(H' - H'_S)/H'_S$. For hexaploid populations of *M. annua*, these statistics were calculated, first, for all populations; second, for each of the two sexual system zones (monoecy and androdioecy) depicted in figure 2; third, for monoecious and androdioecious populations regardless of their sexual system zone; and finally, separately for the different sexual systems present within the androdioecious zone. Significant differences between mating systems and between regions were assessed by randomizing populations among categories.

Comparisons between Different Polyploid Levels

Allelic phenotype diversity statistics (such as H') are not comparable between different allopolyploid levels because they are a function of diversity at multiple independent isoloci; coalescent simulations of a subdivided population with disomic inheritance suggest that the difference in H'_T between polyploid levels depends on the degree of differentiation between isoloci (D. J. Obbard, S. A. Harris, and J. R. Pannell, unpublished data). Because no information about allele frequencies for the different isoloci is available in *M. annua*, comparison of genetic diversity between dioecious (diploid) and monoecious or androdioecious (hexaploid) populations could not be made. It was also not possible to compare estimates of F_{IS} between ploidy levels in a meaningful way because F_{IS} essentially relies on the relative numbers of heterozygotes and homozygotes, which cannot be estimated when isoloci are indistinguishable and allele dosage is unknown. However, computer simulations suggest that if inheritance is disomic, F'_{ST} is almost unaffected by polyploid level (D. J. Obbard, S. A. Harris, and J. R. Pannell, unpublished data). This is because F'_{ST} (like F_{ST}) is effectively a ratio of the diversity due to differences between populations relative to the total diversity. We therefore compared levels of population differentiation among all three sexual systems, regardless of ploidy level, using F'_{ST} calculated with FDASH (D. J. Obbard, S. A. Harris, and J. R. Pannell, unpublished data).

Postglacial Range Expansion and Isolation-by-Distance Effects

Diploid *M. annua* is distributed on an east-west axis across Europe from Israel to Spain, and hexaploid *M. annua* is distributed on a north-south axis from Morocco to central Iberia (Durand 1963). Therefore, to assess the possible effects of postglacial range expansion, allelic richness and H_e (for diploids) and H'_S and observed allele numbers (for hexaploids) were regressed on latitude or longitude, as appropriate (see "Results"). Because the sexual system also varies with latitude in hexaploid *M. annua*, we additionally considered the combined effect of both sexual system and latitude on H'_S, using an analysis of variance.

We used regression analysis of pairwise population differentiation, $F'_{ST}/(1-F'_{ST})$, on the logarithm of geographic distance between populations to identify isolation-by-distance effects (Rousset 1997). This was done for all pairs of dioecious, monoecious, and androdioecious populations at two spatial scales (<200 and <600 km). The significance of isolation-by-distance effects was assessed with Mantel tests, as implemented in FSTAT (Goudet 1995). In addition to the comparison of regional F'_{ST} presented in the preceding section, pairwise measures of population differentiation were analyzed using ANOVA to test whether they differed between sexual systems. Degrees of freedom were conservatively calculated from the numbers of populations analyzed rather than the number of pairwise measures. Regression analyses and other statistical tests were performed using MINITAB (release 12.1).

Results

Dioecious Populations

Total gene diversity (H_T) for all dioecious populations was 0.11; average within-population gene diversity (H_S) for dioecious populations was 0.09. Allelic richness and H_T differed significantly between geographic regions across Europe ($P = .001$; table 1). Allelic richness and genetic diversity were highly correlated with longitude ($P < .0001$ for both measures; fig. 3*a*). The effect was slightly stronger for allelic richness than for diversity ($r^2 = 0.55$ and 0.50, respectively), possibly suggesting a loss of rare alleles from east to west. Genetic differentiation (F_{ST}) over the whole range was 0.21 and did not differ among geographic zones ($P = .128$; table 1).

Monoecious and Androdioecious Populations

Region-wide diversity. Sexual system did not affect total diversity (H'_T; measured as the average number of alleles by which pairs of individuals differ, calculated across populations). Total diversity did not differ between the monoecious and androdioecious zones ($P = .961$; see fig. 2 for the "zone" boundaries), between androdioecious and monoecious populations in the androdioecious zone ($P = .493$; table 2), or between all monoecious and all androdioecious populations, irrespective of zone ($P = .659$; table 2).

Table 1: Genetic diversity and differentiation in dioecious populations of *Mercurialis annua*

	N	A	H_T	H_S	F_{IS}	F_{ST}
Population location:						
West	27	1.218	.086	.063	.061	.258
Central	10	1.422	.126	.116	−.025	.090
East	7	1.594	.167	.149	.073	.167
P	…	.001	…[a]	.001	.303	.128
All populations	44	…	.111	.090	.046	.206

Note: Tabulated values are averages across all eight loci. Allelic richness (A) is corrected for sample size and quoted for a sample of eight individuals. H_S is average Nei's (1987) gene diversity calculated within local populations. H_T is total gene diversity for the specified region. F_{ST} and F_{IS} are Weir and Cockerham's (1984) estimators θ and f, respectively. Significance was assessed by randomization over populations between geographic zones.

[a] P value not calculated by FSTAT.

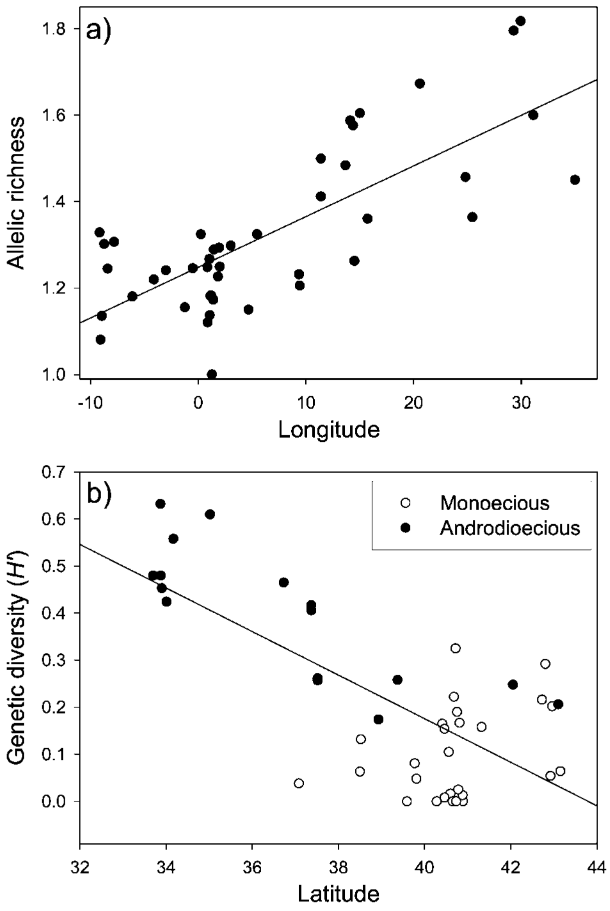

Figure 3: *A*, Average allelic richness per locus (corrected for sample size; El Mousadik and Petit 1996) of diploid populations of *Mercurialis annua* is highly correlated with longitude ($r^2 = 0.55$, $P < .0001$). An almost identical but weaker effect is seen for genetic diversity, H_S (data not shown). *B*, Genetic diversity (H'_S) of hexaploid populations is highly correlated with latitude ($r^2 = 0.50$, $P < .0001$). Monoecious populations from the Canary Islands are not shown because latitude is unlikely to be a good proxy for their migration history; however, if they are included, $r^2 = 0.27$, $P < .001$.

Within-population diversity. Within-population diversity was higher for populations in the androdioecious zone than the monoecious zone, whether diversity was assessed by total number of alleles per population (A; $P < .001$), total number of allelic phenotypes per population (A_p; $P < .001$), or the average number of alleles by which pairs of individuals differed (H'_S; $P < .001$). This was also true for androdioecious populations and monoecious populations within the androdioecious zone, and for all androdioecious and monoecious populations, regardless of geographic zone (table 2).

Population differentiation. Genetic differentiation (F'_{ST}) was higher in the monoecious than in the androdioecious zones (0.63 vs. 0.24; $P < .001$; table 2). It was also higher for monoecious populations ($F'_{ST} = 0.52$) than for androdioecious populations ($F'_{ST} = 0.11$) when zone was disregarded ($P < .001$; table 2). Differentiation across all dioecious (diploid) populations in the Iberian Peninsula was similar to that of androdioecious populations (diploid $F'_{ST} = 0.15$ vs. hexaploid $F'_{ST} = 0.11$). Over distances of up to 600 km, there were no significant effects of isolation by distance. At the smaller spatial scale (<200 km), there was no evidence for isolation by distance between pairs of monoecious populations ($P = .24$; Mantel test) and only a very small effect of geographic distance for pairs of androdioecious populations (gradient = 0.03, $r^2 = 0.23$, $P = .008$; Mantel test) and a small (but marginally insignificant) effect for pairs of dioecious populations (gradient = 0.02, $r^2 = 0.03$, $P = .062$; Mantel test). Differentiation between monoecious populations was higher than between androdioecious populations at all spatial scales ($F = 7.03$, df = 40; $P = .012$) but did not differ between dioecious and androdioecious populations ($F = 1.99$, df = 41; $P = .166$).

The effect of latitude on diversity. Genetic diversity within monoecious and androdioecious populations was negatively correlated with latitude ($r^2 = 0.50$, $P < .0001$; linear regression; fig. 3*b*), as was the number of alleles per population ($r^2 = 0.17$, $P = .003$; linear regression; data not shown). When latitude and sexual system were considered together, both contributed significantly to the variation in diversity (latitude $P = .008$; sexual system $P = .001$; table 3).

Discussion

The distribution of sexual systems within the *Mercurialis annua* species complex has been hypothesized to be the result of a balance between selection for gender specialization and selection for reproductive assurance in a metapopulation (Pannell 2001, 2002; fig. 1). Our study has shown that genetic differentiation is lower between dioecious populations and between androdioecious populations of *M. annua* than it is between monoecious populations. In addition, although there are strong geographic trends in genetic diversity within *M. annua* (fig. 3), within-population diversity in populations lacking males is lower than that in populations displaying full or partial separation of the sexes. Thus, observed population genetic structure is consistent with the metapopulation model for sexual system diversity in *M. annua*. The current distribution of sexual systems and neutral genetic diversity in *M. annua* is also partly the result of postglacial range expansion from at least two different Pleistocene refugia. Below, we consider the likely phylogeographic history of *M. annua* before evaluating the genetic evidence for the

Table 2: Genetic diversity and differentiation in monoecious and androdioecious populations of *Mercurialis annua*

	Total diversity			Within-population diversity						Differentiation	
Populations	*N*	H'_T	*P*	*A*	*P*	A_P	*P*	H'_S	*P*	F'_{ST}	*P*
Monoecious zone	24	.636	.961[a]	2.337	<.001[a]	1.590	<.001[a]	.136	<.001[a]	.629	.003[a]
Androdioecious zone	18	.649		3.253		3.718		.453		.236	
Monoecious populations	4	.695	.493[b]	2.327	.006[b]	1.516	<.001[b]	.112	<.001[b]	.562	<.001[b]
Androdioecious populations	14	.612		3.488		4.279		.528		.106	
All monoecious populations	29	.537	.659[c]	2.098	<.001[c]	1.475	<.001[c]	.111	<.001[c]	.519	<.001[c]
All androdioecious populations	16	.507		3.007		3.576		.426		.109	
All populations	45	.554		2.424		2.227		.217		.436	

Note: Tabulated values are averages across loci, weighted for sample size. *A* is the observed number of alleles, and A_P is the observed number of allelic phenotypes per population. H'_S and H'_T are within-population and total diversity (expressed as the average number of alleles by which pairs of individuals differ). F'_{ST} is calculated as $(H'_T - H'_S)/H'_T$. "All populations" includes samples from the Canary Islands. Significant differences were inferred by randomizing populations between analysis groups (1,000 randomizations).

[a] *P* value for comparison of populations in monoecious and androdioecious zones.

[b] *P* value for comparison of monoecious and androdioecious populations within androdioecious zone.

[c] *P* value for comparison of all monoecious and all androdioecious populations.

importance of metapopulation processes in regulating the distribution of its sexual systems.

Geographic Patterns in Species-Wide Genetic Diversity

Geographic gradients in genetic diversity suggest that the diploid cytotype of *M. annua* expanded its range across Europe from a Pleistocene refugium in the eastern Mediterranean region and that the hexaploid cytotype expanded across the Iberian Peninsula from a north African or southern Iberian refugium. Because rare alleles are expected to be lost during the repeated bottlenecks that occur during range expansion, gradients in allelic richness and genetic diversity can point to refugium sources, where richness and diversity will be highest (Hewitt 1999; Petit et al. 2003). In *M. annua*, our estimates of allelic richness and genetic diversity among diploid populations were highest in eastern Europe and the eastern Mediterranean region and were reduced with increased distance west from this putative refugium source. Similarly, we found highest allelic richness and genetic diversity for hexaploid populations in southern Spain and northern Morocco, with reduced values in more northern populations.

In species whose current distribution is the result of expansion from more than one refugium, diversity hot spots can result from the admixture of alleles where lineages from distinct refugia meet, complicating or obscuring phylogeographic inference (Charlesworth 2003; Petit et al. 2003). Because there has evidently been very little, if any, gene flow between diploid and hexaploid populations in their zones of contact in northeastern and northwestern Spain, the genetic patterns displayed by each cytotype lack the complicating signature of admixture and allow relatively straightforward inference. These clear gradients in genetic diversity and allelic richness displayed by both diploid and hexaploid *M. annua* are similar to those observed in several other species, for example, the cactus *Lophocereus schottii* in Baja California (Nason et al. 2002), and oak gall wasps in Europe (Rokas et al. 2003).

In Europe, cases of postglacial range expansion have been loosely classified into three broad categories (Hewitt 1999). The "hedgehog" model corresponds to expansion into northern Europe from separate refugia in the Iberian Peninsula, Italy, and Greece or Turkey. The "grasshopper" model corresponds to range expansion predominantly from a single refugium in Greece or Turkey, with Iberian and Italian lineages failing to leave their respective peninsulas. Finally, the "bear" model corresponds to expansion from separate refugia in Greece or Turkey and the Iberian Peninsula, with lineages failing to leave the Italian peninsula. In plants, *Quercus* spp. and *Abies alba* match the "hedgehog" scenario, while *Alnus glutinosa* (King and Ferris 1998) and *Fagus sylvatica* (Demesure et al. 1996) match the "grasshopper" scenario. Our study, however, is one of the very few that have allowed phylogeographic inference for a weedy annual (see also Koch and Bernhardt 2004).

Despite sparse sampling in eastern Europe, diploid *M. annua* appears to correspond to the "grasshopper" para-

Table 3: The effects of latitude and sexual system on within-population diversity (H'_S) in hexaploid *Mercurialis annua* in the Iberian Peninsula and Morocco

Source	df	Mean square	*F*	*P*
Latitude	1	.09100	7.84	.008
Sexual system	1	.23485	20.22	<.001
Error	40	.01161		
Total	42			

digm. Although the gradient in allelic richness and genetic diversity in diploid *M. annua* might also be consistent with the prior existence of low-diversity refugia in the west, the similarity in allele frequencies across the diploid range (Obbard 2004) is evidence against this possibility. The observed gradient cannot be attributed to differences in the intensity of sampling because genetic diversity was highest in the east, where sampling intensity was lowest. Neither can it easily be attributed to a gradient in population census size; *M. annua* is common across the entire range that we sampled. Indeed, populations were largest and most common in northeastern Spain (S. M. Eppley and J. R. Pannell, unpublished data), where diversity is particularly low (fig. 3). Finally, it is possible that differences in migration between populations or in the rate of population turnover could account for the genetic gradients observed. However, we found no significant difference in population differentiation between the geographic regions (table 1), which would also be expected under this scenario.

Gradients in allelic richness and genetic diversity in hexaploid *M. annua* in the Iberian Peninsula and north Africa suggest a scenario of range expansion from a refugium in the south; note that these latitudinal genetic gradients remained significant in a multiple regression that also accounted for the possible confounding latitudinal differences in sexual system or (meta-) population dynamics. Importantly, the scenario of a northward range expansion from north Africa is also consistent with the widespread occurrence of polyploid (tetraploid, hexaploid, and octoploid) populations of *M. annua* along the north coast of Africa and on the Mediterranean islands of Corsica and Sardinia (Durand 1963).

These patterns do not correspond to any of the three animal paradigms outlined by Hewitt (1999). This may be because few studies have considered species that are widespread in both north Africa and Europe; however, Michaux et al. (2003) and Harris et al. (2002) viewed African populations of wood mice and wall lizards, respectively, as sinks for migration rather than as sources. One interesting exception is that of *Ecballium elaterium* (the "squirting cucumber"), where separate refugia for distinct monoecious and dioecious races have also been proposed (Costich and Meagher 1992). It seems likely that dioecious *E. elaterium* expanded into Europe from a north African refugium, whereas the monoecious race probably expanded from the east (Costich and Meagher 1992). This is similar to the most likely scenario for *M. annua*, except that sexual system variation in *E. elaterium* does not correlate with ploidy, and the monoecious and dioecious populations of *M. annua* expanded from the south and east, respectively. It is noteworthy that separate glacial refugia for diploid and polyploid races have also been hypothesized for other European plant species (e.g., Trewick et al. 2002; Koch and Bernhardt 2004); such differences in ploidy and sexual system may have been able to arise only under geographic isolation imposed by glaciation.

Evidence for the Metapopulation Model

The metapopulation hypothesis for the distribution of sexual system in *M. annua* (Pannell 1997*a*, 1997*c*, 2001) is summarized in the introduction to this article and illustrated in figure 1. It proposes that monoecy has evolved and is maintained by selection for reproductive assurance in areas where population turnover is high, migration among extant populations is low, and populations are frequently established by single or small numbers of individuals. In contrast, unisexual individuals (either males only or males and females) will be maintained only when the migration rate between extant populations is high relative to the rate of population turnover (Pannell 1997*a*, 2001). Previous observations of sex ratios in androdioecious regions, where highly variable male frequencies might be interpreted as a signature of colonization history, are consistent with the metapopulation hypothesis (for similar ideas in the context of gynodioecy, see also Belhassen et al. 1989; Manicacci et al. 1996). However, ours is the first study to assess evidence for the model by comparing possible genetic signatures of colonization among regions that differ in their sexual systems. Below, we discuss this evidence and evaluate alternative explanations for our observations.

Total genetic diversity within regions. Metapopulation dynamics are predicted to cause a reduction in the total genetic diversity maintained in a species if the population turnover rate is greater than the rate of migration amongst extant populations (Slatkin 1977; Pannell and Charlesworth 1999). However, we found no evidence for this prediction in comparisons between regions of hexaploid *M. annua* with different sexual systems and under putatively different population turnover rates. In particular, total genetic diversity ($H_T^{\prime}$) was the same in monoecious and androdioecious zones, for monoecious and androdioecious populations regardless of zone, and for monoecious and androdioecious population in the androdioecious zone (table 2).

Regional and species-wide comparisons of genetic diversity are notoriously difficult to interpret in terms of processes operating within regions (Charlesworth and Pannell 2001). This is largely because the models assume a metapopulation-wide equilibrium, whereas equilibration of absolute measures of metapopulation-wide diversity can be very protracted (Pannell and Charlesworth 1999). The difficulty arises also because of the "closed-system" assumptions of the models. Thus, if a high rate of population turnover occurs in a region that receives migrants from adjacent regions that suffer lower turnover rates, regional comparisons can be obscured. This scenario is very likely in *M. annua*, where steep gradients often occur between sexual systems and between regions with very different demographic structures (S. M. Eppley and J. R. Pannell, unpublished data). Absolute measures of genetic diversity are therefore likely to be more informative about the deeper phylogeographic history of lineages over wide geographic scales, as discussed above, than about regional processes.

Within-population diversity. As in the case of metapopulation-wide diversity, theory also predicts greatly reduced genetic diversity under high population turnover rates within populations as a result of local genetic bottlenecks during colonization (Pannell and Charlesworth 1999; Wakeley and Aliacar 2001; Pannell 2003). However, unlike predictions for metapopulation-wide diversity, the prediction for the effect of metapopulation processes on within-population diversity is made with reference to means over multiple populations, none of which are assumed to be at an equilibrium. Thus, patterns of within-population diversity are more robust indicators of metapopulation processes than total genetic differentiation (Pannell and Charlesworth 1999). In accordance with predictions of the metapopulation model for sexual system variation in *M. annua*, our estimate of within-population diversity (H'_S) was significantly lower for populations in monoecious regions than in androdioecious regions. It was also lower for monoecious populations, regardless of region, as well as for monoecious populations in the androdioecious zone (table 2). These differences were robust to the possible confounding effects of latitude (and thus, phylogeographic history).

It is well established that within-population diversity also depends on the mating system, with predominantly selfing populations typically less diverse than those that are outcrossing (Hamrick and Godt 1996; Charlesworth and Pannell 2001; Charlesworth et al. 2003; Pannell and Dorken 2006). In particular, for loci with disomic inheritance, as in our study, the diversity of a population with selfing rate S is expected to be equal to $(1 - S/2)$ times the diversity of an equivalent outcrossing population (Pollak 1987). Thus, the differences in within-population diversity between regions with different sexual systems might be due to differences in their rates of inbreeding rather than metapopulation dynamics. However, outcrossing rates in established hexaploid *M. annua* populations are probably greater than 0.5 and may be much higher (S. M. Eppley and J. R. Pannell, unpublished data). The fourfold reduction in genetic diversity observed within monoecious compared with androdioecious populations thus cannot be explained by differences in the selfing rate alone (see table 2).

Genetic differentiation. Genetic differentiation between populations, quantified by F_{ST} or equivalent polyploid statistics, is a particularly good measure of population structure, especially for slowly evolving loci like allozymes. This is because its value gets close to equilibrium much more quickly than the component absolute measures of within-population and metapopulation-wide diversity, of which it is a ratio (Pannell and Charlesworth 2000). Moreover, F_{ST} is largely independent of the absolute diversity in the metapopulation and of the mutation rate (as long as both of these are low), so it can be used directly to compare metapopulations or regions with different evolutionary histories (Charlesworth 1998; Nagylaki 1998; Pannell and Charlesworth 2000).

In hexaploid *M. annua*, F'_{ST} was much higher for monoecious populations than for androdioecious populations. This difference was apparent at all spatial scales analyzed in our survey and thus cannot be attributed to an artifact of sampling. Metapopulation genetic theory predicts that population turnover should increase F_{ST} if the average number of migrants moving between extant populations is less than twice the number of individuals that first colonized the population (Wade and McCauley 1988; Whitlock and McCauley 1990). This condition will be met if colonization and postcolonization gene flow result from the same process of dispersal, as is the case for passive seed dispersal (Wade and McCauley 1988). However, elevated levels of genetic differentiation are also expected to be found in selfing relative to outcrossing species because selfing reduces the population effective size (Pollak 1987; Ingvarsson 2001); it may also be associated with reduced migration rates through pollen dispersal, as well as increased susceptibility to the effects of local genetic hitchhiking or background selection (Charlesworth and Pannell 2001; Charlesworth 2003; Pannell and Dorken 2006).

It is unlikely that the elevated value of genetic differentiation in monoecious populations of *M. annua* is due to hitchhiking or background selection. These processes occur as a result of strong linkage disequilibrium between positively or negatively selected loci and the marker loci used to measure diversity. Sufficiently strong linkage disequilibrium can be maintained across the genome only for very high rates of self-fertilization. However, selfing rates are likely to be high only during the very early stages of population establishment in *M. annua*, and they quickly fall to low levels in the dense stands typical of the populations that we sampled (S. M. Eppley and J. R. Pannell, unpublished data). It is more likely that the observed differences in population differentiation are due to the combined differential effects of colonization and population turnover on the one hand and migration among extant sites on the other.

The possible interaction between migration and the sexual system presents perhaps the greatest source of ambiguity in interpreting observations of population structure in *M. annua.* Because androdioecious populations contain males, which disperse between four and 10 times more pollen than monoecious individuals (Pannell 1997*c*), we should expect gene flow among androdioecious populations to be higher, and genetic differentiation lower, than between monoecious populations, as observed. This would suggest that population genetic structure might be due to differences in gene flow rather than differences in population turnover. Although gene flow among extant populations is indeed likely to play an important role in regulating population structure, it is unlikely to be a sufficient explanation for our observations. This is because genetic differentiation was high not only among monoecious populations in monoecious regions but also within androdioecious regions, where we might have expected gene flow from androdioecious populations to have had a homogenizing influence. The most parsimonious explanation is therefore that monoecious populations in androdioecious regions were more recently colonized than androdioecious populations, as supposed in the metapopulation model.

Conclusions

Taken together with our phylogeographic analysis, population and regional comparisons of genetic diversity implicate both ancient and ongoing colonization as key factors influencing the distribution of combined versus separate sexes in *M. annua.* Our study thus joins ranks with several others that point to the importance of regional processes on patterns of sexual system variation in plants (reviewed in Pannell and Dorken 2006), although none of these studies considered the evolution of combined versus separate sexes in systems that span the complete range from hermaphroditism to dioecy. A metapopulation model has also been invoked to account for the maintenance of androdioecy in several independent plant and animal lineages (Pannell 2002), but ours is the first study to test this hypothesis empirically.

Although the metapopulation concept has been extremely influential in the study of animal populations, the prevalence of metapopulation dynamics in plants is still controversial (Freckleton and Watkinson 2002, 2003; Ehrlén and Eriksson 2003; Pannell and Obbard 2003). A key criticism of the applicability of metapopulation theory for plants is that they often do not occupy discrete habitat patches. This criticism applies in particular to species such as *M. annua*, whose populations can be diffuse and extensive along the disturbed verges of country roads (Freckleton and Watkinson 2002). Given observed variation in sex allocation and sex ratios in androdioecious regions, demographic signatures of population turnover in terms of variation in population size and occupancy (S. M. Eppley and J. R. Pannell, unpublished data), and the patterns of population genetic structure reported here, the evidence for an effect of metapopulation dynamics on sexual system variation in *M. annua* is compelling. Of course, much of this evidence is indirect, and the model's ultimate test will require direct measures of population turnover in natural populations. To this end, we have begun a long-term study to monitor the dynamics of approximately 500 populations of *M. annua* across several transitions of its sexual system throughout its range in north Africa and the Iberian Peninsula.

Acknowledgments

We thank R. J. A. Buggs, M. E. Dorken, and S. M. Eppley for valuable discussion and two anonymous reviewers for useful comments on the manuscript. We are grateful to the following people for providing samples of *M. annua* used in this study: R. Buggs, G. Campbell, S. Eppley, L. Fontes, A. Golan, P. Hamouz, M. Harris, T. Mitchell, K. Obbard, S. Obbard, E. Pacini, T. Rayden, P. Schlüter, F. Topliffe, R. Wesselingh, and E. Williams. D. J. Obbard was supported by a Long Studentship from Queens College, Oxford, and the research was partly supported by grants to J. R. Pannell and S. A. Harris from the Natural Environment Research Council of the United Kingdom.

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