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Widespread functional androdioecy in *Mercurialis annua* L. (Euphorbiaceae)

JOHN PANNELL[1]

Department of Plant Sciences, Oxford University, Oxford, OX1 3RB

The widespread coexistence of male and monoecious (cosexual) plants in Spanish, Portuguese and Moroccan populations of *Mercurialis annua*, an annual wind-pollinated ruderal, represents an important case of functional androdioecy, a rare breeding system in plants and animals. In *M. annua*, both males and cosexes disperse fully competent pollen. Quantitative gender varies discontinuously between males and cosexes, with males producing a mean of 6.09 times as much pollen as cosexes. It appears that gender is determined by a simple developmental switch, with male and cosexual inflorescences differing markedly in morphology: staminate flowers are borne on erect peduncles in males and in tight spiral clusters around a subsessile pistillate flower in cosexes. Males do not differ from cosexes in their biomass, but they are significantly taller, principally as a result of their greater internode lengths. The cosexual inflorescence is strongly protogynous so that outcrossing is favoured in dense stands, but seed-set is assured in cosexes isolated from prospective mates because of their ability to self-fertilize. Males typically occur at frequencies of less than about 30% in androdioecious populations, in accordance with theoretical predictions for functional androdioecy. In the genus *Mercurialis*, dioecy is the ancestral condition and monoecy and androdioecy, which occur in polyploid populations of *M. annua*, are derived. I argue here that androdioecy is most likely to evolve in plants (1) from dioecy, (2) in wind-pollinated species, and (3) in species with a colonizing habit. These predictions are also consistent with the limited published data available for other species.

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ADDITIONAL KEY WORDS:—dioecy – gynodioecy – reproductive assurance – Baker's Law – mating system – wind pollination – Mediterranean – sex ratio.

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[1]Current address: Department of Botany, University of Toronto, 25 Willcocks St Toronto, Ontario, M5S 3B2 Canada. E-mail: pannell@botany.utoronto.ca

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INTRODUCTION

Androdioecy denotes the coexistence of males and hermaphrodites in a population (Darwin, 1877). It is a particularly rare breeding system in plants which continues to attract interest whenever a new possible case is investigated (Thomson *et al.*, 1989; Liston, Rieseberg & Elias, 1990; Fritsch & Rieseberg, 1992; Lepart & Dommee, 1992; Rieseberg, Hanson & Philbrick, 1992; Philbrick & Rieseberg, 1994). Several models of androdioecy have been published (Lloyd, 1975b; Ross & Weir, 1976; Charlesworth & Charlesworth, 1978; Charlesworth, 1984; Pannell, 1997b), and they predict the rarity of androdioecy relative to gynodioecy (the coexistence of females with cosexes). In an outcrossing population, the evolution of gynodioecy and androdioecy are equally likely if gender is determined by nuclear genes, the condition being that both males and females are at least twice as fertile as the corresponding sexual function of the hermaphrodites (cosexes). However, in a partially selfing population in which selfed progeny suffer from inbreeding depression, the fertility requirement for females decreases whereas it increases for males. This is because males must compete for access to fewer outcrossing ovules, while females may produce fitter, obligately outcrossed progeny. This asymmetry, alongside the rarity of androdioecy, has been cited as evidence for the importance that selection for outcrossing has had in the evolution of dioecy (Charlesworth & Charlesworth, 1978; Thomson & Barrett, 1981), although alternative hypotheses based on ecological factors (Bawa, 1980; Givnish, 1980, 1982; Bawa & Beach, 1981) and sexual selection (Willson, 1991) have also been ventured.

Just over a decade ago, Charlesworth (1984) reviewed the then published cases of morphological androdioecy in plants, and she concluded that all of them were probably examples of functional dioecy or cosexuality rather than androdioecy. Either: (1) the morphological cosexes were functional females with dysfunctional anthers or sterile pollen (suggesting that their pollen may be serving as a pollinator reward and that the breeding system is cryptically dioecious; Charlesworth, 1984; Schlessman, Lowry & Lloyd, 1990; Cane, 1993a); (2) males were always small and apparently later became cosexual; or (3) cryptic dioecy was betrayed by a 1:1 sex ratio (Mayer & Charlesworth, 1991).

Since Charlesworth's (1984) review, several new potential cases of androdioecy have come to the fore. Thomson *et al.* (1989) found a few male individuals in a

small, otherwise cosexual population of *Ricinocarpos pinifolius* in southeastern Australia. The population met Charlesworth's (1984) criteria for functional androdioecy, but the authors acknowledged that androdioecy may not be an appropriate term, as the frequency distribution of floral gender was not discontinuous or bimodal; instead, quantitative gender (Lloyd, 1979, 1980) varied continuously from pure male through to strongly female. Lloyd & Bawa (1984) referred to this type of gender distribution as simply 'asymmetric'. They commented that this asymmetry, which may be quite common in simultaneous cosexes, is incorrectly described as androdioecy, suggesting that the term be reserved for cases where males are a clearly distinct gender from cosexes (Lloyd & Bawa, 1984).

Lepart & Dommee (1992) studied two morphologically androdioecious populations of *Phillyrea angustifolia* in southern France, and they found that cosexes were only partially successful as male parents in one population and functionally female in the other. They concluded that *P. angustifolia* presented a case of 'leaky dioecy'. Traveset (1994) studied two Spanish populations of *P. angustifolia* and argued that they represented functional androdioecy, though in one of them gender was distributed continuously between males and cosexes. She also commented that the congeneric *P. latifolia* may be androdioecious, though to date there are no detailed data for this species (but see Aronne & Wilcock, 1994). Cane (1993b) found that in a morphologically androdioecious species of *Saurauia* in Costa Rica, pollen from pistillate flowers was infertile, and he classified the breeding system as cryptically dioecious.

Only *Datisca glomerata*, a riparian herbaceous perennial, has so far been widely heralded as a functionally androdioecious species (Liston *et al.*, 1990; Fritsch & Rieseberg, 1992; Rieseberg *et al.*, 1992). In *D. glomerata*, males occur at a frequency of less than 25% and produce 3.8 times as many fertile anthers as cosexes (Liston *et al.*, 1990). Outcrossing rates accord with levels required for the maintenance of androdioecy by selection as predicted by theory (Fritsch & Rieseberg, 1992). Accepting the significance of androdioecy in *Datisca glomerata*, it is nevertheless very limited in its extent; the ten populations assessed by Liston *et al.* (1990) were all small (only one of them exceeding 100 individuals) and half of them contained no males at all. Given the interest that androdioecy in *Datisca* has attracted recently, it is therefore all the more surprising that the far more widespread occurrence of androdioecy in *Mercurialis annua* L., a common European ruderal, has remained unstudied and has been overlooked in all reviews of plant breeding systems (although it is cited briefly by Richards [1986]).

THE SPECIES

In a biosystematic study of the polyploid complex in *M. annua*, Durand (1963) described populations in southern Spain and northern Morocco which comprised males, at frequencies of less than 50%, co-occurring with monoecious individuals (Fig. 1). He described these populations as androdioecious and showed that the monoecious plants were self-compatible. Durand's (1963) morphological descriptions clearly point to a discontinuity in sex expression between males and monoecious morphs, though he made no assessment of phenotypic gender distributions within populations, as defined by Lloyd (1979, 1980; Primack & Lloyd, 1980).

Mercurialis is a European and Mediterranean genus in the Euphorbiaceae with
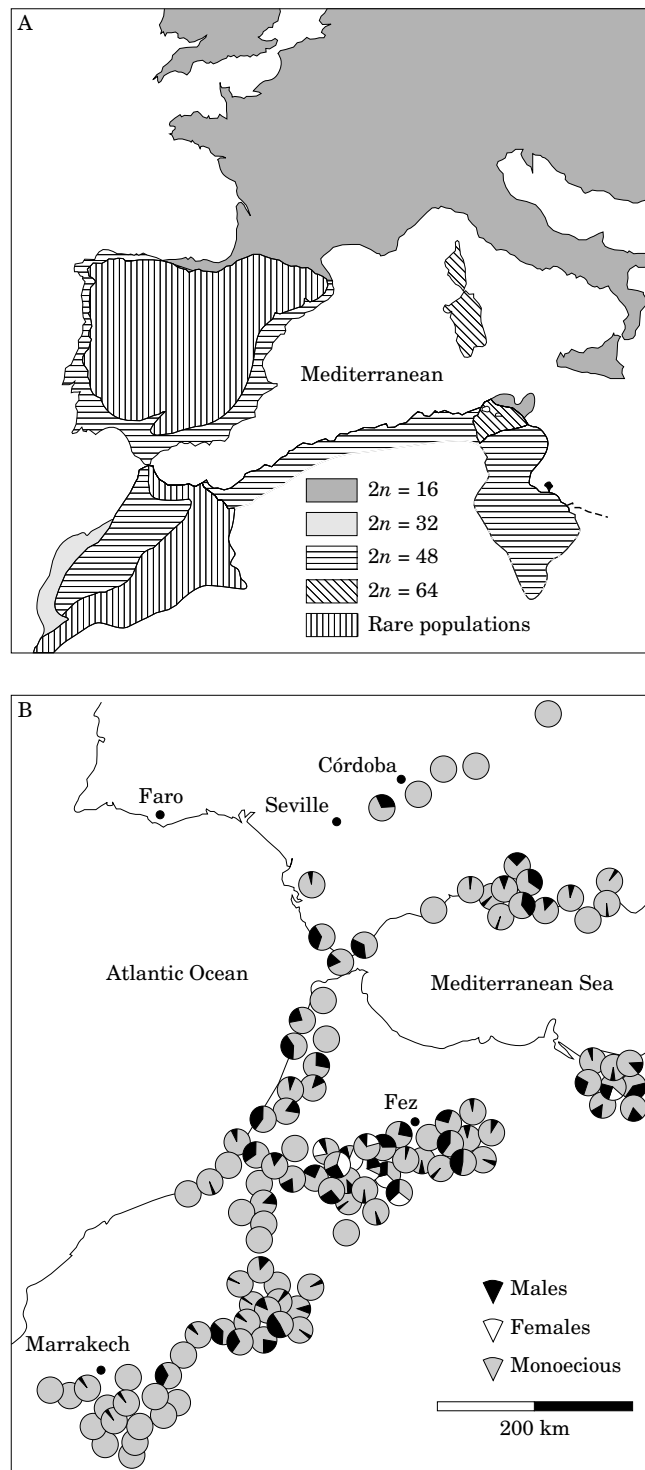
Figure 1. A, the distribution of *Mercurialis annua* in western Europe; B, hexaploid monoecious and androdioecious populations sampled by Durand (1963) in southern Spain and northern Morocco. Adapted, with permission, from Durand (1963).

seven recognized species (Tutin *et al.*, 1968; Valdes, Talavera & Fernandez-Galiano, 1987). All these are wind-pollinated, and all but *M. annua* are dioecious perennials. *M. annua* is an herbaceous annual. Over most of its distribution (north-west of Barcelona throughout central Europe), it is diploid ($2n = 16$) and dioecious (Fig. 1). The diploids give way to monoecious polyploid populations ($4n$ to $12n$) south of Barcelona on the Iberian Peninsula, as well as in Morocco, Tunisia, Corsica and Sardinia (Durand, 1963). The androdioecious populations of *M. annua* in southern Spain, southern Portugal and northern Morocco are all hexaploid (Durand, 1963; Durand & Durand, 1992).

On an initial survey of the region sampled by Durand (1963), I confirmed the widespread occurrence of androdioecy in the Algarve in southern Portugal, throughout southwestern Andalucía in Spain, and as far south as Marrakech in Morocco. Here, populations are frequently dense, may form nearly monospecific stands and occur at wasteland sites disturbed by humans, e.g. along road-sides, amongst ruins, around rubbish dumps, and as garden invaders. The present study was initiated to characterise their breeding system more precisely and to decide whether it presents a significant case of functional androdioecy in plants.

MATERIAL AND METHODS

Study sites

Four sites were chosen for study within the metropolitan area of Seville in Andalucía, southern Spain: (1) in the precincts of the Pabellón de Cuba (PC site); (2) in a large garden bed of the Parque de María Luisa (ML site); (3) on the banks of the Río Guadalquivir beside the Generalísimo bridge (RG site); and (4) beside the Informática car park of the University of Seville (CP site). At each site, *M. annua* formed a dense stand and was by far the most dominant species, though the RG population was shaded by trees. The field work was carried out in February and March of 1994 (all sites but CP) and 1995 (RG and CP).

Sex ratios and size polymorphism

At each site, adjacent transects of contiguous quadrats were laid two to four meters long through the stand of *M. annua*. Quadrats were either 0.5×0.5 m (where the stand was particularly dense) or 1.0×1.0 m in size. In each quadrat, all plants were harvested at ground level and taken back to the lab for assessment. Numbers of males, cosexes and immature plants yet to flower were recorded for all quadrats. For quadrats in some of the transects (see results), plants were tallied for height classes in 0.10 m increments. For two quadrats at the PC site and one at the RG site, all plants were measured individually for both height and fresh weight. In all, 75 quadrats were harvested and assessed. The mean biomass of male and cosex individuals in each quadrat was estimated by dividing their combined dry weight (plants dried at 60° for 48 h) by their number in the sample. The difference between male and cosex estimates was analysed for significance by a two-tailed *t*-test using data from all 75 quadrats.

Phenotypic gender assessment

Six large males and cosexes were chosen haphazardly from the CP site and returned to the lab where they were placed in dry vases at room temperature. From each plant, 10 staminate flowers at the point of dehiscence were removed and placed together in an aluminium foil crucible in which they were dried for 24 h at 60°C and weighed. Ten staminate flowers which had already dehisced and dispersed all their pollen (but which had not yet been abscised from the plant) were dried and weighed similarly. Pollen weight per mature flower was estimated for each plant as the difference between the mean full flower and empty flower dry weights.

Between 10 and 30 males and cosexes (see results) were chosen randomly from each of several quadrats at each site. For each plant, its height, its dry mass (plants dried at 60°C for 48 h), and the number of nodes on its main stem were recorded. All staminate flowers were removed from each plant with fine forceps and their combined dry weight was recorded (flowers dried in aluminium foil crucibles at 60°C for 24 h). For cosexes, the number of two-seeded and three-seeded fruits was also recorded. The quantitative gender of individual plants in each population (Lloyd, 1979, 1980) was calculated in terms of their prospective femaleness, $G_i = g_i/(g_i + a_i.E)$, where g_i is the number of seeds on the plant, a_i is the dry weight of staminate flowers, and E is an equivalence factor which equates the prospective contribution of the measured female and male reproductive units in the population (Lloyd, 1980). E was calculated as the average seed production per individual in the population divided by the average individual production of staminate flowers, or equivalently as $E = S.(1-F)/\{P_m.F + P_c.(1-F)\}$, where S is the number of seeds produced per biomass by cosexes, P_m and P_c are the dry weights of staminate flowers produced per biomass by males and cosexes, respectively, and F is the frequency of males in the population. Cosexes were ranked according to their G_i, and quantitative gender was then plotted against its rank in the sample (Lloyd, 1980).

Pollen size and viability

Six males and six cosexes were selected haphazardly from the CP site. A further six cosexes were chosen from a completely monoecious population in the Sierra de Córdoba, north-east of Seville. The pollen from a mature anther from each plant was twice washed in a drop of ethanol on a microscope slide, mounted in glycerol gel and sealed under a cover-slip with paraffin, following the methods of Cushing (1961). The polar (a) and equatorial (b) radii of six grains from each plant were recorded, and their volume was estimated as $4\pi ab^2/3$, the volume of a prolate ellipsoid (Weast & Selby, 1975). Variances were homogeneous, and the raw data were analysed by a two-factor Anova, with individual plants nested in the gender class.

Pollen viability was estimated indirectly by its capacity to metabolise the fluorescent dye, fluorocine di-acetate (FDA). Pollen from a mature anther from each of 10 males and 10 cosexes, grown from seed sampled in 1993 from a population of *M. annua* about 50 km north-west of Faro in southern Portugal, was mounted in a solution of FDA in a 1% sucrose buffer and left for 10 minutes at room temperature.

Approximately 200 pollen grains were scored for their fluorescence. Arcsine-transformed data were analysed by a one-factor Anova; variances were homogeneous after a single outlier had been removed.

Breeding system

Seed collected in March, 1993, from an androdioecious population near Fez, Morocco, was germinated in June, 1993, and seedlings were grown under glasshouse conditions in Britain. Before the onset of flowering, but after characteristic male and cosexual inflorescences had begun to develop, 20 cosexes were isolated individually in large, pollen-proof boxes. (The boxes measured 0.60×0.40 basally and 0.50 m in height and comprised wooden frames covered above and on two sides by PVC clear plastic and on the other two sides by the breathable, pollen-proof material widely used in the construction of pollination bags.) Five individually isolated cosexes were used as controls: every three days, all staminate flowers were removed with sterilised fine forceps before they had dehisced; the pistillate flowers were left intact. Five isolated cosexes were allowed to self-pollinate. Five cosexes, each repeatedly emasculated as described above, were paired with another cosex which was allowed to produce and disperse pollen. Finally, five emasculated cosexes were each paired with a male individual which produced and dispersed pollen. Every 3 days, for 3 weeks after plants began dispersing pollen, observations of the seed set by each cosex were recorded. (The experiment was discontinued at three weeks due to pollen contamination; see Results).

RESULTS

Description of floral and fruit biology

Inconspicuous pistillate flowers are produced in the leaf axils (leaves are opposite) on short pedicels which seldom exceed 5 mm in length when fruits are mature (Fig. 2). Their three green tepals are already open when formed. The superior ovary consists of three locules, each with a single ovule, though typically only two of them develop. The stigmas are feathery, usually measuring about 1.5 mm in length but increasing in size to over 3 mm if they are not pollinated. Each fruit produces usually two, but occasionally three, seeds; single-seeded fruits almost never occur, and all pistillate flowers set seed. (The only plant found with much less than maximum seed set was an isolated individual with almost completely sterile anthers; extensive searching in its vicinity failed to locate any other *M. annua* plants.) Thus in natural populations, seed production is probably not limited by pollen availability. Seeds develop rapidly and are dispersed by ballistic fruit dehiscence about two weeks after pollination.

In cosexual plants, between 5 and 15 closed staminate flowers are produced in a tight spiral cluster at the base of each pistillate flower (Fig. 2). In male plants, flowers (indistinguishable from the staminate flowers in cosexes) are produced in similar tight clusters at irregular intervals along an erect, axillary peduncle which may be up to 0.80 m in length (Fig. 2). Each flower has three green tepals. The staminate
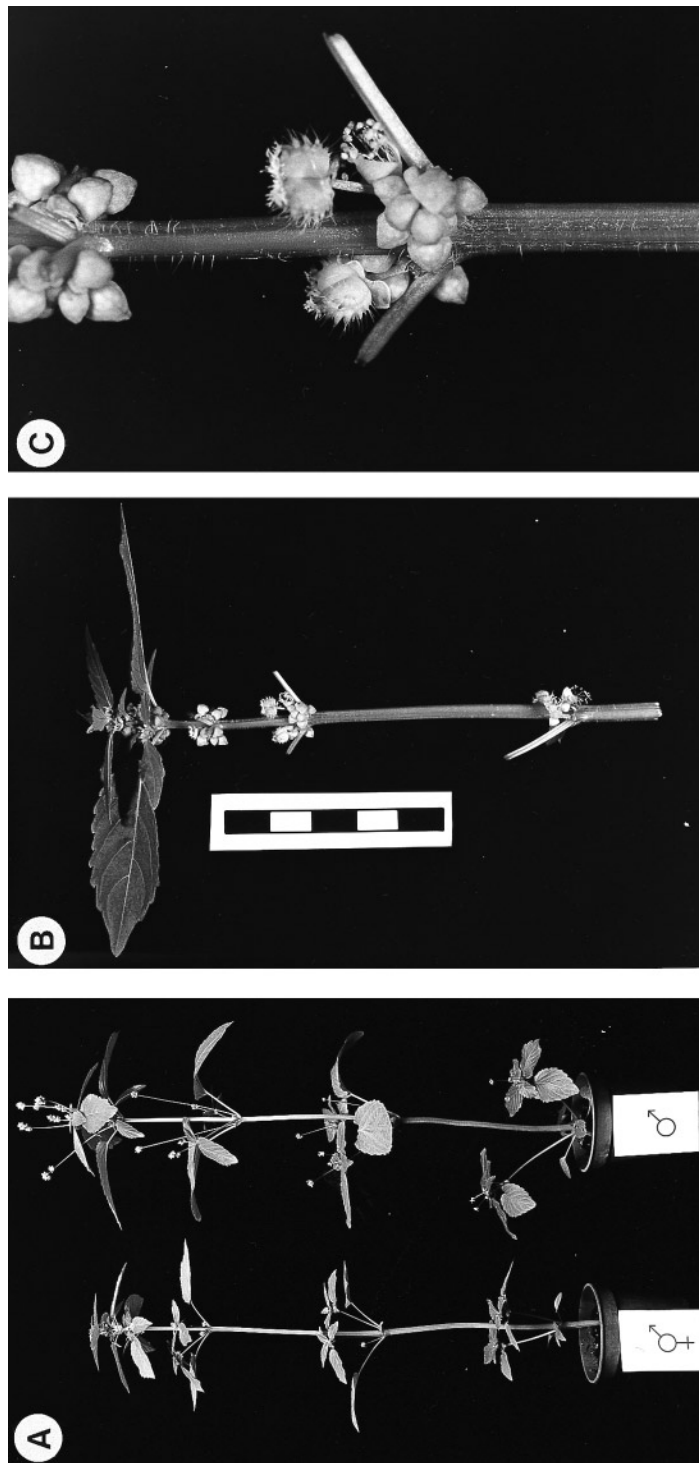
Figure 2. A, monoecious and male individuals of *M. annua*; the lables are 10 cm high. Note the erect pedunculate inflorescences on the male plant. B, monoecious stem apex with the lower leaves removed to show the position of the axillary inflorescences; the scale bar shows 1 cm increments. C, close-up of a monoecious inflorescence.

TABLE 1. Means and standard errors of several pollen and plant size characters for males and cosexes in populations around Seville. [a] $n = 6$ plants; see Anova table in Table 2; mean volume of pollen grains from the monoecious population was 42.02 ± 1.82 nm³; [b]Anova on arcsine-transformed data, $F = 0.39$, d.f. = 1, 17 (1 outlier removed); [c]Anova: $F = 14.9$, d.f. = 1, 11; [d]Anova: $F = 14.8$, d.f. = 1, 11; [e]Anova: $F = 16.45$, d.f. = 1, 245; [f]Calculated as plant height/number of nodes on primary stem, Anova: $F = 75.27$, d.f. = 1, 245; [g]two-tailed t-test of (cosex − male) biomass difference in 75 quadrats, in which there was a mean of 47.8 ± 3.3 males and 271 ± 17 cosexes per quadrat (the mean biomass difference was 0.0191 ± 0.022 g).

Variable	Male	Cosex	P
Pollen volume (nm³)[a]	33.45	38.58	$= 0.108$
	(2.00)	(2.11)	
Pollen viability (%)[b]	80.2	84.1	$= 0.54$
	(11.9)	(9.28)	
Pollen biomass/flower (μg)[c]	246.7	315.0	$= 0.003*$
	(16.9)	(5.63)	
Pollen biomass/flower biomass[d]	0.554	0.557	$= 0.91$
	(0.027)	(0.008)	
Plant height/biomass (mm/g)[e]	1777	1165	$< 0.001*$
	(11.9)	(9.35)	
Internode length (mm)[f]	75.8	64.2	$< 0.001*$
	(0.098)	(0.008)	
Plant biomass[g]			$= 0.38$

TABLE 2. Anova of pollen grain volume from male and cosexual plants from an androdioecious population and from cosexual plants from a monoecious population. There were six plants per gender and six pollen grains measured per plant

Source	d.f.	MS	F	P
Gender	2	502.3	3.48	0.057
Plant (Gender)	15	144.2	5.86	<0.001
Error	90	24.6		
Total	107			

flowers in each spiral inflorescence develop, open, disperse their pollen, and are then abscised from the plant sequentially, usually with only one or two open at any one time. At dehiscence, the tepals spring back and expose 10 to 20 closed anthers. Anthesis occurs within minutes, and all pollen is dispersed to the wind. Cosexual inflorescences are strongly protogynous, and stigmas have usually been pollinated, with seeds at an advanced state of development, before the first staminate flower in the inflorescence dehisces.

Pollen size, viability and breeding system

There were no significant differences in the size of pollen grains between males and cosexes in the androdioecious population and cosexes in the monoecious population (Tables 1 and 2). Pollen grains did not differ significantly between males and cosexes in their capacity to metabolize FDA (Table 1) and are thus apparently equally viable.

TABLE 3. Seed-set recorded on cosexual plants isolated in pollen-proof growth boxes. Plants were scored every 3 days after the onset of flowering. x marks boxes in which the cosexes had set seed (once seed had set, no further emasculation was carried out in that box). The Control contained only emasculated plants; Selfing plants were isolated on their own; the 'x Cosex' treatment contained an emasculated cosex together with an unemasculated cosex; the 'x Male' treatment contained an emasculated cosex together with an unemasculated male

Treatment	Box	3-day intervals					
		1	2	3	4	5	6
Control	1						xxx
	2				xxx	xxx	xxx
	3					xxx	xxx
	4[a]						
	5					xxx	xxx
Selfing	1		xxx	xxx	xxx	xxx	xxx
	2	xxx	xxx	xxx	xxx	xxx	xxx
	3						
	4[a]						
	5	xxx	xxx	xxx	xxx	xxx	xxx
x Cosex	1	xxx	xxx	xxx	xxx	xxx	xxx
	2	xxx	xxx	xxx	xxx	xxx	xxx
	3[a]						
	4		xxx	xxx	xxx	xxx	xxx
	5	xxx	xxx	xxx	xxx	xxx	xxx
x Male	1	xxx	xxx	xxx	xxx	xxx	xxx
	2	xxx	xxx	xxx	xxx	xxx	xxx
	3	xxx	xxx	xxx	xxx	xxx	xxx
	4	xxx	xxx	xxx	xxx	xxx	xxx
	5	xxx	xxx	xxx	xxx	xxx	xxx

[a]In these boxes, the cosex was apparently sterile and failed to produce any pollen over the three weeks of the experiment (see text).

The wind-pollination syndrome, combined with continued floral production in new leaf axils throughout the growing season, made it difficult to prevent uncontrolled pollination from occurring in the growth boxes. After about 2 weeks, three of the emasculated cosexes had set seed, apparently through self-pollination from a staminate flower I had missed (Table 3). A similar margin of error can be assumed in the other treatments where emasculation was carried out. However, notwithstanding this limitation to the experiment, the differences between the control and the remaining treatments leave little doubt that *M. annua* is self-compatible and that ovules can be fertilized by both males and cosexes as found by Durand (1963). Interestingly, in three of the boxes, the cosexes turned out to be producing anthers with no pollen or sterile pollen. (The inviability of the pollen was confirmed by its inability to metabolize FDA.) These plants were kept in isolation for several weeks after the experiment was terminated, and all three plants began producing viable pollen later in their growing season and set seed.

Consistency of sex expression

Males and cosexes are morphologically distinct in their inflorescence architecture and are easily scored for gender. Moreover, although fruits and staminate flowers are abscised from the plants after pollen and seed dispersal, respectively, the characteristic peduncles and pedicels persist until plant death so that the consistency of sex expression in an individual can be assessed. I examined tens of thousands of plants in the study area and found sex expression to be well conserved in individuals: plants with male (pedunculate) inflorescence do not produce pistillate flowers. Occasionally, I found cosexes with one or two male pedunculate inflorescence in the first or second leaf axils. This would suggest that a small fraction of the plants classified as cosexes started out with entirely male function and would have been classified in this study as males when still small. (Pannell [1997a] found that this cosexual phenotype, which had produced early male inflorescences, were actually modified genetic males. However, because the breeding system is defined in terms of sexual phenotypes rather than genotypes, they are appropriately classified here as cosexes.) Certainly from the time the second leaf pair had been formed, plants remained either consistently male or cosexual. In the spring of 1994, I observed the development of plants at the RG site carefully until their death at the onset of the summer drought, and there was no alteration in sex expression to the end of the season. This observation was confirmed with plants growing under glasshouse conditions.

Significantly, I found one large aberrant individual which *had* haphazardly varied the gender of its branches. Primary branches from the principal stem were either entirely male (with the characteristic male inflorescences only and producing no pistillate flowers at all), or entirely cosexual (lacking the male peduncles and producing sessile cosexual inflorescences in the leaf axils). This strongly suggests that sex determination in *M. annua* is controlled by a developmental switch between a male and a cosexual pathway and that phenotypic gender is *qualitatively* different between males and cosexes.

Androdioecious sex ratios and gender size distributions

Table 4 presents the sex ratios found in the studied populations in Seville, with individuals summed across quadrats at each site. Males occurred at a frequency of less than 25% at all sites except for CP which had about 38% males. Very few plants had not reached sexual maturity when they were counted (Table 4). Between- and within-site variation in sex ratios and sex allocation in *M. annua* are considered in more detail in Pannell (1997c).

I found no evidence for differences in mean above-ground dry weight between males and cosexes, despite the large number of quadrats assessed and the number of individuals per quadrat over which means were estimated (Table 1). However, males were about 1.5 times taller for their mass than cosexes (Table 1), a fact which can be accounted for by greater internode lengths along the principal stem in males (Table 1). Figure 3 shows the size distribution of males and cosexes in a quadrat at the GB site (the distributions were similar in the other quadrats measured). Cosexes had a much more widely spread height distribution than did males (Fig. 3A), with males peaking sharply in frequency in the higher classes. The distribution of fresh

TABLE 4.(a) Number of males, cosexes, immature (non-flowering) individuals, and percentages of males in populations of *M. annua* in and around Seville (percentages ignore immature plants). Numbers are from combined quadrats at each site; a, b and c refer to more or less isolated 'sub-populations', separated at PC by less than 10 m and at GR by about 30 m. Populations were sampled in February and March of 1994; * denotes sites sampled in 1995 as well. (b) Combined numbers of males, cosexes and immature individuals from seven quadrats at PC; individuals greater than 0.30 m and less than 0.30 m in height were recorded separately

Site	Males	Cosexes	Immature	% Males
(a)				
PC.a	461	1936	37	19.23
PC.b	1016	7092	202	12.53
PC.c	315	2162	14	12.72
ML	365	1658	23	18.04
NS	94	404	0	18.88
GR.a	145	869	48	16.69
GR.a*	277	1747	11	15.86
GR.b	288	1491	59	19.32
GR.b*	355	1523	18	23.31
CP*	120	319	0	37.60
(b)				
>0.30 m	178	689	0	20.53
<0.30 m	177	1342	31	11.65

weights was highly skewed to smaller plants in cosexes, while the frequency of male fresh weights peaked in the intermediate range (Fig. 3B).

Relative male fecundity and phenotypic gender

Cosexes produced significantly larger staminate flowers than did males (Table 1). However, the proportion of floral biomass accounted for by pollen was almost identical in males and cosexes (55%; Table 1). This confirms that comparisons in staminate floral biomass between males and cosexes give a reliable estimate for relative male fecundity, as they closely reflect pollen productivity.

Figure 4 shows the staminate floral dry weight plotted against plant dry weight for males and cosexes from sites PC and GB, respectively; similar observations were made at the other sites. Floral production varied linearly with plant dry weight for both genders, although cosexes displayed greater variation for plants of the same biomass than did males. Importantly, relative male fertility did not vary continuously between males and cosexes for plants of the same size but was clearly distinct. For each of the 17 assessed quadrats, the regression coefficient of staminate floral biomass on above-ground plant dry-weight was calculated for males and cosexes (31 of the 34 regressions were significant at $P = 0.001$, the remaining three were significant at $P = 0.01$; r^2 values ranged from 0.46 to 0.99). The ratio of the male to cosex regression coefficients, which is an estimate of relative male fertility controlling for plant size, was 6.09 ± 0.73 (mean $\pm$ SE; $n = 17$) and ranged from 2.33 to 15.5.
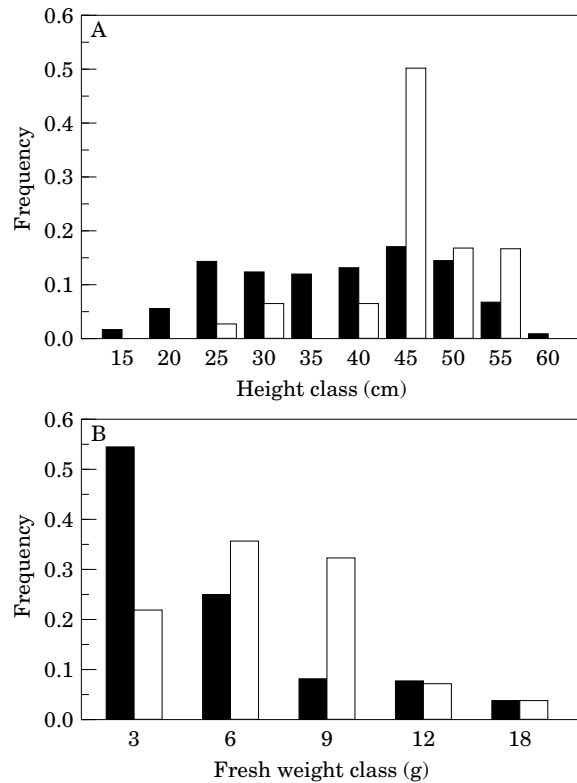
Figure 3. The distribution of (A) heights and (B) fresh weights in a quadrat at the GR site for males and cosexes. Frequencies are standardized so that they sum to one for each sex. (■) Cosex; (□) male.

The distribution of phenotypic gender (prospective femaleness) is shown in Figure 5 for cosexes sampled from a quadrat at each of sites PC and GR (males produced no seed and therefore had a gender of zero). Although there is wide variation in gender in cosexes, from about 0.6 to 1.0, there is clearly a large discontinuity between their gender value and that of males. Much of the variation in cosexual gender is associated with differences in plant size. This is illustrated clearly in Figure 5: smaller cosexes are quantitatively more female that larger ones.

<div align="center">DISCUSSION</div>

<div align="center">*Functional androdioecy confirmed for* M. annua</div>

There is no doubt that *Mercurialis annua* is functionally androdioecious. Previous putative cases of androdioecious plant species have almost invariably turned out to be functionally dioecious or cosexual (Charlesworth, 1984; Thomson *et al.*, 1989; Cane, 1993b). Others are simply cases of gender inconsistency (Lepart & Dommee, 1992) or particularly wide gender adjustments (Lloyd & Bawa, 1984; Thomson *et al.*, 1989). Functional androdioecy in *M. annua*, however, cannot be discounted on
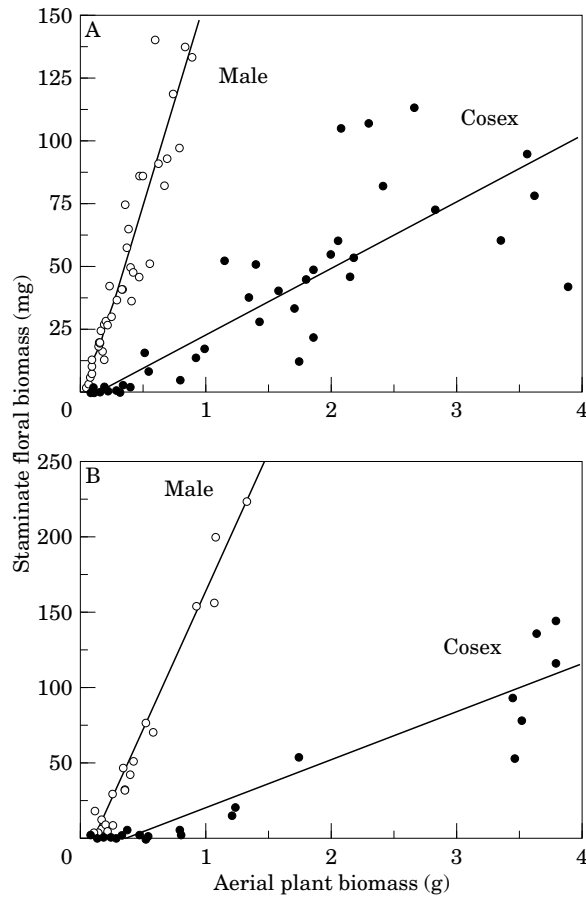
Figure 4. The staminate floral biomass plotted against the above-ground biomass of males and cosexes (A) from two adjacent quadrats at the PC site, and (B) from a single quadrat at the GR site.

any of these grounds. Cosexes disperse fully competent pollen and are thus not functionally female, and males retain their unisexual function throughout their life and are not smaller in biomass than cosexes, as is found in other putatively androdioecious species (Charlesworth, 1984).

Theory predicts that in a functionally androdioecious population, males will be less frequent than cosexes and more than twice as fertile in their pollen function (Lloyd, 1975b; Charlesworth & Charlesworth, 1978). In androdioecious populations of *M. annua*, males occur consistently at low frequencies (usually <30%) and produce a mean of 6.09 times more pollen than cosexes, well in accord with these expectations. Models of androdioecy predict the frequency of males in a population at equilibrium in terms of relative male fertility, the selfing rate of the cosexes and the level of inbreeding depression suffered by selfed progeny (e.g. Charlesworth & Charlesworth, 1978). Although we have no estimates of selfing rates and inbreeding depression for *M. annua*, Figure 6 indicates the range of their values which would be consistent with Charlesworth & Charlesworth's (1978) model for an androdioecious population
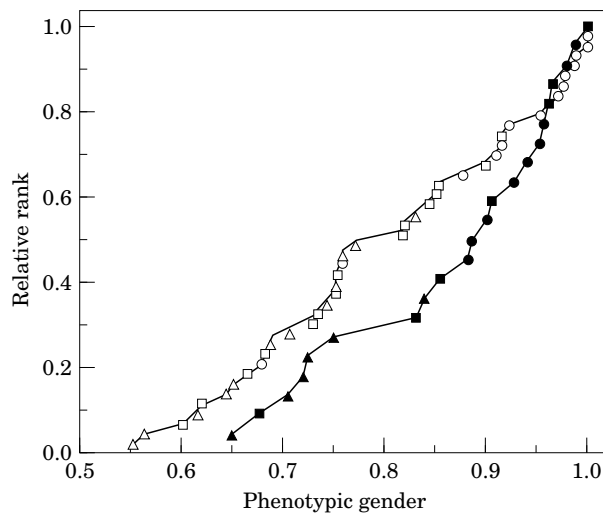
Figure 5. The phenotypic gender of cosexes sampled at the PC site (open symbols) and the RG site (closed symbols), plotted in order of its rank in the sample. Ranks have been standardized for each site to lie between zero and one. Plants of different sizes are indicated by the symbols. (○,●)<0.5 g; (□,■)<2.0 g; (△,▲)<4.0 g.
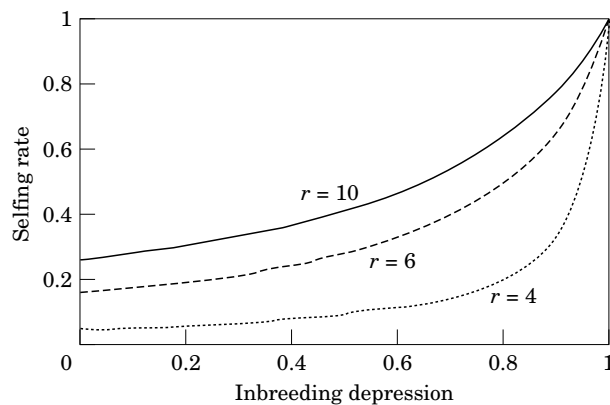

Figure 6. Values of the rate of self-fertilization and inbreeding depression which would be consistent with the model for androdioecy of Charlesworth & Charlesworth (1978), assuming that males occur in an equilibrium population at a frequency of 0.3. Three curves are given, with r, the relative male fertility (amount of pollen dispersed by males relative to cosexes), $=10$, 6 and 4. (Populations of *M. annua* had a mean r of 6.09; see text.)

at equilibrium in which males occur at a frequency of 0.3 and disperse four, six and ten times more pollen than cosexes (i.e. values typical for *M. annua*).

Finally, males are both qualitatively as well as quantitatively distinct from cosexes in their phenotypic gender. Their different inflorescence structure and greater height/biomass ratio underscore the qualitative gender distinction. Furthermore, the discovery of a plant which varied the gender of its branches between either fully male or fully cosexual implies a qualitative developmental switch determining gender.

Gender differences in *M. annua* are thus not merely the result of quantitative gender adjustments. Indeed, Pannell (1997a) found that maleness in androdioecious *M. annua* was genetically determined by a dominant allele at a single locus, although environmental factors (such as reduced stand density) caused some genetic males to function as cosexes.

Unisexuality in Mercurialis

Almost certainly, dioecy was the ancestral condition in *Mercurialis*, and it is plain that monoecy in the polyploid populations of *M. annua* is derived within the species and genus; all other six species in the genus are dioecious, with the only exception being the monoecious polyploid populations of *M. annua* (Tutin *et al.*, 1968; Valdes *et al.*, 1987). Indeed, in the Euphorbiaceae as a whole, a cosmopolitan family with over 300 genera and 7500 species, all species have unisexual flowers and many are dioecious (Cronquist, 1981). The evolution of androdioecy in *M. annua* has therefore not required the evolution of unisexuality (maleness) *de novo*; whatever the actual path taken to androdioecy, the novel step was the evolution of *cosexuality*, not unisexuality. Thus we should endeavour to understand androdioecy in *M. annua* as having evolved from long established dioecy, not from functional hermaphroditism as theoretical have attempted to do (Lloyd, 1975b; Charlesworth & Charlesworth, 1978; Charlesworth, 1984). As these models have shown, androdioecy is unlikely to evolve from cosexuality as a response to selection for outbreeding because the conditions for the invasion of males in a cosexual population become more severe with increasing rates of self-fertilization.

The importance of wind pollination

The sexual dimorphisms in *Mercurialis* strongly suggest that the syndrome of wind pollination has been important in maintaining unisexuality in the genus. This is not only evident in the divergent morphology of the male and female inflorescence throughout the genus (Tutin *et al.*, 1968), but also in the secondary sexual height dimorphism in *M. annua*, where males are taller and more slender than cosexes (this study) and than females in dioecious populations (Durand & Durand, 1991). It would appear that male architecture in *M. annua* has been selected for the dispersal of pollen and that both females and cosexes have been selected to maximize pollen capture by stigmas (Niklas, 1985). Similar inter-gender differences in architecture have been summarized for other species by Bickel & Freeman (1994) and de Jong & Klinkhamer (1994), and they are likely to be important in maintaining a separation of the sexes in wind-pollinated plants in general (Givnish, 1982; Charlesworth, 1993).

It is important to note that the sexual dimorphisms in *M. annua* will affect the functional gender of individual plants. The functional female gender of a plant is its realized gender in terms of the proportion of progeny it produces as a maternal parent (Lloyd & Bawa, 1984). This study characterized the quantitative gender of individuals in terms of their relative seed and pollen output. Lloyd & Bawa (1984) pointed out that although the phenotypic and functional gender are closely related, "they are not identical if pollen and seeds from different sources vary in their

probable or realised success". In *M. annua*, we see a rather extreme example of this, where pollen produced by males is likely to enjoy greater outcrossing success than that produced by cosexes, due to both the inflorescence and the height dimorphisms. Thus, measures of the relative male fecundity, although already high, should be regarded as a conservative estimate of male fitness. I suggest that this may be one important reason for the maintenance of males with cosexes in androdioecious populations.

Cosexuality in M. annua

While diploid *M. annua* is dioecious, plants in polyploid populations are monoecious and self-compatible. Such changes with polyploidy from obligate outbreeding systems to facultative or obligate self-fertilizing systems are common, and several possible hypotheses have been suggested for this trend (Stebbins, 1985; Thomson & Lumaret, 1992; Ward *et al.*, 1994). One possible explanation is that the harmful genetic effects of selfing, such as the loss of heterozygosity or the expression of deleterious recessive alleles (Lande & Schemske, 1985; Schemske & Lande, 1985; Agren & Schemske, 1993) may be mitigated in polyploids. It has been suggested, for example, that heterozygosity may become fixed in polyploids through the process of the diploidization of the genome (Sybenga, 1969; Thomson & Lumaret, 1992).

What might have given rise to the evolution of cosexuality in *M. annua*? Durand (1963) synthesized polyploids from dioecious diploid *M. annua* and found in the F$_2$ progeny a mixture of males, females and monoecious individuals of similar morphology to those found in natural populations. He suggested that the process of polyploidization had led to a disruption in the balance of sex-determining alleles, and that subsequent selection had led to the fixation of monoecy in natural polyploids. Durand cited the distribution of unisexuality in *M. annua* tetraploids and hexaploids as support for this hypothesis: hexaploid populations frequently contain males, whereas tetraploids, which supposedly predate hexaploids and have had more time to respond to selection, are almost completely monoecious (Durand, 1963). This argument is weakened when one considers that all higher ploidy levels ($2n>48$) in *M. annua* are exclusively monoecious. I suggest, rather, that we regard the distribution of breeding systems in *M. annua* as adaptive within the normal limits of developmental and trade-off constraints, and not as constrained by a putative lack of time for adaptive evolution.

Durand (1963) suggested that cosexuality would be favoured in *M. annua* in the semi-arid Mediterranean environment, because of the ability of single seeds to colonize new sites on their own. This hypothesis for the selective role of reproductive assurance in colonizing species (Piper, Charlesworth & Charlesworth, 1986; Jarne & Charlesworth, 1993; Pannell, 1997b) was first emphasized by Baker (1955), and it has frequently been referred to as 'Baker's Law' (Baker, 1967; Charlesworth & Charlesworth, 1979; Holsinger, 1986). Thus, cosexuality may be maintained in polyploid populations of *M. annua* by selection for facultative selfing when colonizing new sites, a possibility which is consistent with the high degree of progogyny in the cosexual inflorescence. This should favour outcrossing when outcross pollen is available, and the close proximity of staminate and pistillate flowers in the monoecious inflorescence should assure seed-set through selfing when cosexes find themselves isolated from prospective mates. In theoretical metapopulation models, Pannell

(1995; 1997b) found that recurrent population extinction and recolonization dynamics could maintain cosexuality in a species in which dioecy would otherwise be stable in long-lived populations. It would appear that this situation applies to *M. annua*.

Why androdioecy rather than gynodioecy?

Theoretical studies (Lloyd, 1975b; Charlesworth & Charlesworth, 1978; Charlesworth, 1984) and deductions from empirical observations (Lloyd, 1975a) implicate gynodioecy as the most likely path between cosexuality and dioecy. Why then should androdioecy have played this role in *M. annua*?

There are at least three plausible reasons as to why cosexuality might spread in a dioecious population: (1) to avoid the genetic cost of outcrossing (Fisher, 1930); (2) as a cost-sharing exercise, whereby cosexual plants use the same resources for organs which serve both sex functions with less than double the investment (e.g. organs for pollinator attraction; Charnov, Maynard Smith & Bull, 1976; Lloyd, 1988); and (3) to assure reproductive success during colonization (Baker, 1955; Baker & Cox, 1984; Jarne & Charlesworth, 1993), as mentioned above.

The first situation is unlikely to lead to androdioecy. If self-fertilizing cosexes successfully invaded a dioecious population, they would almost certainly spread to fixation, or, if not, gynodioecy rather than androdioecy might persist (we do not expect males to persist in a population with selfing cosexes). Neither should we expect the second situation to occur in a wind-pollinated species like *M. annua* in which no reproductive costs are shared between the male and female sexual functions. In animal-pollinated species, this situation might lead to the evolution of androdioecy only in species which remain outcrossing and where pollen is the pollinator reward; where pollinators are rewarded with nectar, cost-sharing would imply gynodioecy as the path to cosexuality rather than androdioecy.

The third situation is most plausible for the evolution of androdioecy, especially in a wind-pollinated species like *M. annua*. Cosexuality is most likely to evolve in the sex in which outcrossing success would be least compromised by bet-hedging for reproductive assurance. If wind-pollinating males began producing pistillate flowers, their ovules would almost certainly be fertilized by selfing, since the local pollen cloud around their stigmas would comprise mainly self pollen (Levin & Kerster, 1974). Furthermore, a shift in inflorescence architecture to accommodate pistillate flowers and fruits is likely to reduce the outcrossing success of males. This is most obviously implicated in *M. annua* in the contrast between male and cosexual inflorescences, where staminate flowers on males are well positioned for pollen dispersal, while those on cosexes are not (Niklas, 1985; and see Figure 2).

A further reason why we might expect the production of a few pistillate flowers by males to be an inefficient or unsuccessful adaptation for reproductive assurance is that the number of progeny they could produce at a new site would be small, and colonization may frequently fail as a result. This contrasts with the situation where females produce a few staminate flowers, as their continued relatively high seed production would place them in a much more favourable position to establish a new population on their own. The production of pollen by female individuals should not necessarily compromise their outcrossing efficiency in dense stands, particularly with strong protogyny, as occurs in *M. annua*.

The distribution of androdioecy in M. annua

In *M. annua*, there are no dioecious diploid populations in Iberia or around the western Mediterranean; all are polyploid and monoecious or androdioecious. Within this region, androdioecious populations occur in the better watered regions on either side of the Strait of Gibraltar. In the drier areas further west and north on the Iberian Peninsula and further south in Morocco, all populations are monoecious. The influence of a climatic gradient on the vegetation of this region has been well documented (Emberger, 1939; Sauvage, 1961; Durand, 1963): the subhumid areas in which androdioecy occurs in *M. annua* are occupied characteristically by vegetation associations of *Quercus suber*, which are absent in the drier zones where only monoecious populations of *M. annua* occur. It is significant that in the more humid, higher area around Meknes and Fez in central northern Morocco, where we might expect selection for reproductive assurance and cosexuality to be weakest, the hexaploid populations are described by Durand (1963) as 'almost dioecious'. Some of these populations contain both males and females co-existing with cosexes (Durand, 1963, and pers. observ.).

CONCLUSIONS

Current models for the evolution of androdioecy find that unisexual males are unlikely to be able to spread in a cosexual population. I have argued here, however, that androdioecy may evolve in its own right, or as a path to cosexuality, from a dioecious state under conditions not explicitly addressed in these models. The likelihood of the evolution of androdioecy is thus context-dependent. I have also argued that by considering the different modes of selection which may favour a reversion from dioecy to cosexuality (i.e. as avoidance of the cost of sex, as a cost-sharing enterprise, or as an adaptation to reproductive assurance), we place ourselves in a good position to evaluate the likelihood of a path to cosexuality via androdioecy or gynodioecy. Such reasoning predicts that androdioecy is most likely to evolve (1) from dioecy, such that unisexuality does not need to evolve *de novo*; (2) as a response to selection for reproductive assurance in a colonizing species; and (3) in a wind-pollinated species, where selection for separation of the sexes in maintaining dioecy will have been strongest.

This prediction holds true for *M. annua* in all three points. In the only other well studied androdioecious species, *Datisca glomerata*, androdioecy is thought to have evolved from dioecy (Rieseberg *et al.*, 1992). *D. glomerata* is a perennial herb which colonizes disturbed riparian sites, and its status as a colonizer could thus be regarded as intermediate. It is also wind-pollinated. Marginally androdioecious populations of *Phillyrea angustifolia* and *P. latifolia* are wind-pollinated and also inhabit disturbed habit (Lepart & Dommee, 1992; Traveset, 1994). Traveset (1994) believed that androdioecy in these species is more likely to have evolved from hermaphroditism, but it is perhaps significant that unisexual flowers are well established in the family (Oleaceae) in the form of andromonoecy (Traveset, 1994). If we turn to the animal kingdom, we find that although androdioecy is also rare, it does occur in the notostracan *Eulimnadia texana* (Sassaman, 1989, 1991). Androdioecy in this species, which establishes ephemeral populations in non-perennial lakes and ponds, favours

outcrossing when males are present but assures reproductive success during colonization of new habitat in a way closely analogous to the situation described here for plant species.

All these species bear out the predictions for androdioecy outlined in this paper. The fact that there are so few androdioecious species, however, does not mean that androdioecy has necessarily been a correspondingly rare pathway from dioecy to cosexuality. In *M. annua*, androdioecious populations appear to be restricted in their distribution to a region where environmental conditions are intermediate between those favouring cosexuality and those favouring unisexuality. It is thus rather fortuitous that androdioecy has 'been preserved' in this way for us to observe. Conceivably there are other similar cases where androdioecy has appeared and disappeared again as an ephemeral intermediate stage in the transition between unisexuality and cosexuality.

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