Variation in Sex Ratios and Sex Allocation in Androdioecious Mercurialis Annua

John Pannell

Variation in sex ratios and sex allocation in androdioecious *Mercurialis annua*

JOHN PANNELL

Department of Plant Sciences, Oxford, OX1 3RB, United Kingdom

Summary

1 Strong spatial heterogeneity in male frequencies and allocation to male and female function was found within and between androdioecious populations of the wind-pollinated ruderal *Mercurialis annua* in south-western Spain.

2 Sex determination is largely genetic and the constancy from year to year of the frequency of males in small quadrats suggests that seed and pollen dispersal are very limited.

3 Male frequency correlated significantly with stand density in two populations in the field and varied nonrandomly between densities in a glasshouse density experiment, strongly suggesting an environmental component to sex determination.

4 In the density experiment, stand density had a strong negative effect on relative allocation to reproduction by both males and cosexes (monoecious individuals). At higher densities, cosexes were quantitatively more female than at lower densities. Also, larger males and cosexes produced more pollen per vegetative biomass than did smaller ones, while allocation to seed production by cosexes increased only proportionally with plant biomass, so that cosexual gender shifted consistently towards greater maleness in larger plants.

5 Males produced between about five and 10 times as much pollen as cosexes, indicating that cosex sex allocation is strongly female-biased. This, and inflorescence architecture, suggest that female-biased cosexuality in *M. annua* has evolved as a response to selection for facultative self-fertilization in a wind-pollinated species.

6 In *M. annua*, androdioecy appears to be maintained by a balance between selection for reproductive assurance during colonization, which favours self-fertile cosexuality with female-biased sex allocation, and frequency-dependent selection within populations, favouring increased male allocation and thus allowing the invasion and spread of males.

Keywords: androdioecy, dioecy, reproductive assurance, trade-off, wind pollination

Journal of Ecology (1997) **85**, 57–69

Introduction

A central focus of sex allocation theory has been its attempt to explain the distribution of dioecy vs. hermaphroditism (Charnov *et al.* 1976; Charnov 1982; Lloyd 1987b). It predicts that in outcrossing populations hermaphroditism (cosexuality) will be stable if the fitness gained by allocating resources to both sexual functions is greater than that gained by the allocation of all reproductive resources to only one sex, and dioecy will be stable if the converse is true (Charnov *et al.* 1976; Charnov 1982).

Present address: Department of Botany, University of Toronto, 25 Willcocks St. Toronto, Ontario, Canada M5S 3B2.

Factors which might favour cosexuality in a population include low resource overlap, the sharing of fixed costs between male and female sexual functions, local mate competition, and local resource competition (Charnov *et al.* 1976; Charnov 1982; Lloyd 1983, 1984; Lloyd & Bawa 1984; Bulmer 1986; Willson 1990; Bickel & Freeman 1994). Low resource overlap may arise when different resources limit pollen and fruit production, or when investment in each sexual function is separated temporally (e.g. fruit production occurs after male investment has been completed). This should be unfavourable to a separation of the sexes, as a reduction in allocation to one sex does not make resources available to the other (Charnov *et al.* 1976). Cost-sharing between male and

female functions is most likely to occur in insect-pollinated plants with hermaphroditic flowers, where investment in organs for attracting, manipulating and rewarding pollinators benefits both male and female fitness components. Local competition between pollen grains from the same or a related parent for access to ovules (local mate competition; Hamilton 1967) reduces the benefit to individuals with high allocation to male function, while competition between related seed progeny for limited resources (local resource competition) will reduce the fitness gain of individuals with high female allocation.

The above ideas are well established in the theoretical literature, but they are difficult to test empirically because species are usually *either* cosexual *or* dioecious, so that direct comparisons cannot readily be made (Charlesworth & Charlesworth 1981; Barrett 1992). One approach has been to assess ecological and life-history trends among dioecious and cosexual species (e.g. Willson 1979; Bawa 1980; Givnish 1980; Bawa & Beach 1981; Donoghue 1989; Thomson & Brunet 1990). Another approach has been to study species in which unisexual and cosexual individuals are present in the same population.

Gynodioecious species have provided fertile ground for such research. Several studies have found evidence for 'compensation' (Darwin 1877), where male-steriles, which produce no pollen, increase their seed production (reviewed in Ashman 1994), while others have found little difference in allocation patterns to seed production between male-steriles and cosexes (van Damme & van Delden 1984). In this second group, it is thought that male-steriles might persist in a population through enhanced survivorship, presumably by increasing their allocation to tissue maintenance, growth and respiration (van Damme & van Delden 1984). Alternatively, gynodioecy may be maintained if females produce fitter progeny than cosexes. This possibility is underpinned by theory which predicts that male-steriles in a partially selfing cosexual population can invade and spread without increased seed production if inbreeding depression sufficiently reduces the fitness of selfed progeny (Lloyd 1975; Charlesworth & Charlesworth 1978). More importantly perhaps, cytoplasmically inherited male-sterility can be maintained in a population even if females are only marginally fitter than the female function of the cosexes (Frank 1989).

Because gynodioecy may be maintained for reasons other than those associated with resource use (i.e. through the avoidance of inbreeding depression or if sterility is inherited cytoplasmically), it would seem that it is actually not an especially good breeding system for testing theories of sex allocation. Androdioecy, however, does not suffer from this drawback; for males to invade and spread in a cosexual population, they must *always* be more than twice as fit as the male function of the cosexes (Lloyd 1975; Charlesworth & Charlesworth 1978; Charlesworth

1984). Androdioecy is thus a much more useful system for testing hypotheses on sex allocation. It also means that studies of sex allocation in androdioecious populations are likely to lead more directly to an understanding of what maintains the breeding system than is the case for gynodioecy.

Unfortunately, the extreme rarity of androdioecy (Charlesworth 1984) has until recently all but precluded such studies, and sex allocation in those few species now shown to display it (*Datisca glomerata*: Liston *et al.* 1990; Fritsch & Rieseberg 1992; Philbrick & Rieseberg 1994; *Phillyrea angustifolia*: Lepart & Dommee 1992; Traveset 1994; and *Mercurialis annua* Pannell 1995, 1997a) has not yet been studied in any detail.

Pannell (1995, 1997a) showed that the coexistence of males and monoecious (cosexual) individuals in southern Iberian and northern Moroccan populations of *Mercurialis annua* are functionally androdioecious. In particular, I noted high inter- and intrapopulation variation in androdioecious sex ratios. In this paper, I examine the spatial variation in male frequencies within and between several populations of *M. annua* in and around Seville in south-western Spain, as well as the temporal variation between two successive generations. I also assess variation in biomass allocation to male and female reproductive functions and examine the possible reasons for such variation. The field data suggested that stand density may be an important factor influencing sex allocation, and on this basis I conducted a controlled glasshouse density experiment to examine this further. A key aim in these studies was to focus specifically on patterns of sex allocation in an attempt to answer how androdioecy is maintained in *M. annua*.

Materials and methods

STUDY SPECIES

Mercurialis annua L. *s.l.* (Euphorbiaceae) is a ruderal annual colonizer of pan-European distribution. Diploid (2n = 16) populations are exclusively dioecious and are widespread across central and western Europe. Polyploids occur around the western Mediterranean in southern Europe and north Africa and are largely monoecious (Durand 1963; Durand & Durand 1992). Apart from *M. annua* polyploids, all seven species in the genus *Mercurialis* are dioecious, and cosexuality would thus appear to be a derived trait (Tutin *et al.* 1968; Valdes *et al.* 1987). In southern Spain, southern Portugal and northern Morocco, hexaploid androdioecious populations of *M. annua* are widespread, with males co-occurring with monoecious plants (cosexes) at frequencies of less than about 30% (Durand 1963; Pannell 1995, 1997a).

In androdioecious populations of *M. annua*, males have the same inflorescence morphology as those of the diploid populations (they have sessile staminate

flowers arranged in tight spiral clusters along erect axillary peduncles). Hexaploid cosexes are similar in morphology to diploid females, except that their sub-sessile axillary pistillate flowers are surrounded basally by a tight spiral cluster of staminate flowers. Both males and cosexes produce inflorescences and fruits indeterminately in their leaf axils, usually from the age of two weeks after germination until they die at the end of the growing season (Pannell 1995, 1997a). In both males and cosexes, the biomass of pollen dispersed accounts consistently for about 55% of the staminate floral biomass (males: $55.4 \pm 2.7\%$; cosexes: 55.7 ± 0.8; Pannell 1997a). Thus staminate floral biomass is a reliable index for allocation to pollen production and is used as such in this study.

STUDY SITES

Nine sites were chosen for study in and around Seville in Andalucía, southern Spain: (1) in the precincts of the Pabellón de Cuba (PC site); (2) along the base of a brick wall about 50 m from the PC site (ME site); (3) beside the Informática car park of the University of Seville (CP site); (4) on the banks of the Río Guadalquivir beside the Generalísimo bridge (RG site); (5) in a large garden bed of the Parque de María Luisa (ML site); (6) on a gravel embankment beside arable land on the northern outskirts of the city (FS site); (7) as for (6), but ≈ 500 m away (AC site); (8) on a gravel roadside embankment on the southern outskirts of the village Aznalcóllar, about 30 km north-west of Seville (AZ site); and (9) in disturbed roadside vegetation on the western outskirts of Aznalcóllar (ES site). The field work was done in February and March in 1994 (all sites but CP) and 1995 (RG and CP).

SEX RATIOS

At each site, adjacent two to four metre-long transects of contiguous quadrats were laid through the stand of *M. annua*. Quadrats were either 0.50×0.50 m (where the stand was particularly dense) or 1.0 m $\times 1.0$ m in size, so that at least 100 plants could be tallied per quadrat. 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.

The seeds produced by cosexes in each of four contiguous 1-m² quadrats at each of the ML and GR sites were retained and grown up under glasshouse conditions in Britain the following summer. The seed from each separate quadrat was mixed, and 12 random samples of 100 seeds each were sown in loam 10 mm beneath the surface in self-draining pots (200 mm in diameter). The pots were laid out randomly on the glasshouse bench on a 0.60-m × 0.60-m grid and were watered daily until plants were harvested 10 weeks after sowing. The number of males and cosexes harvested from each pot was recorded,

as was the combined dry weight of plants of each sex. Sex ratios and male frequencies were calculated for each quadrat by combining data from all 12 replicate pots, and these were compared between quadrats and against the parental sex ratios from the corresponding quadrats in the field. The same procedure was carried out with seed sampled in March 1992 from purely monoecious populations south of Marrakech in Morocco. (See results for analysis.)

ASSESSMENT OF QUANTITATIVE GENDER AND SEX ALLOCATION

Between 10 and 30 males and cosexes were chosen randomly from each of several quadrats at each site. The dry mass of each plant was recorded (plants dried at 60°C for 48 h). All staminate flowers were removed from each plant with fine forceps and their combined dry weight per plant 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 and, in populations sampled in 1995, the dry weight of fruit were also recorded. The quantitative gender (femaleness) of individual plants in each population (Lloyd 1979, 1980) was calculated as $G_i = g_i/(g_i + a_i E)$, 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 for field populations as the average seed production per individual in the population divided by the average individual production of staminate flowers (Lloyd 1980), 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. G_i gives an estimate of the quantitative gender of a plant, because the transmission of genes through either sexual function will depend on the sexual status of the rest of the population. Generally, E may differ between populations, but here it was calculated as a mean value across the populations (or experimental density treatments; see below) to be compared. This is because we are interested here in differences in sex allocation rather than in the effect of changes in E between populations. (See results for analysis.)

THE EFFECT OF SHADING

At the GR site, some patches of *M. annua* were in deep shade, while others were in full sunlight. As a preliminary study to assess the possible effect of shading on sex allocation in the species, three random quadrats were laid out in both the shaded and unshaded patches of the stand, and 10 individual males and cosexes were randomly selected from each. The

number of seeds, the dry fruit biomass, and the cosex and male staminate floral biomass production (all measures were per plant biomass) were analysed by a nested ANOVA, with random quadrats nested in the shaded/unshaded contrast.

DENSITY EXPERIMENT

Seed collected from the PC site in March 1994 was thoroughly mixed and sown in loam at seven different densities 10 mm beneath the soil surface in free-draining pots of 200 mm diameter. The pots were each sown with either 1280, 640, 320 or 160 seeds (four pots of each density), 80 seeds (8 pots), 40 seeds (16 pots) or 20 seeds (32 pots). This meant that at least 640 seeds were sown at each density to ensure that there were sufficient numbers of plants for precise estimates of sex ratios. The pots were laid out randomly on a glasshouse bench and were watered daily until plants were harvested 10 weeks after sowing. All plants from each pot were harvested at ground level, and the numbers of males, cosexes and immature (nonflowering) plants were recorded. For densities where more than four replicates had been sown (20, 40 and 80 seeds per pot), plants harvested from separate pots were lumped together in such a way that there were effectively four replicates per density treatment from which to subsample. Twenty cosexes and 10 males were randomly chosen from each of these four replicates, and their biomass, their number of

seeds and the dry weight of their staminate flowers were recorded as for the field work. Quantitative gender was calculated for cosexes as described above for the field quadrats. (See results for analysis.)

Results

FIELD STUDY

Spatial variation in sex ratios: Within the study region in and around Seville, male frequencies varied widely between sites, from less than 1% at the AC and ES sites to just under 30% at the CP site (see weighted means in Table 1). Frequency variation was pronounced even within small areas: for example, in Area 1 in Table 1, frequencies varied across distances of less than 20 m from 5.43% to 19.5%. In Areas 5 and 6, sites separated by less than 500 m varied in their male frequencies from almost zero to 16.6% and 24.1%, respectively. Although all sites were well delineated and supported apparently homogeneous stands of *M. annua*, male frequencies within them were often significantly heterogeneous across distances of one to four metres (Table 1).

Temporal variation in sex ratios

Compared with the spatial variation cited above, there was little temporal variation in male frequencies from one year to the next, both when plants from the

Table 1 Within- and between-site variation in the frequency (percentage, presented as means with ranges in parentheses) of males in sample quadrats. For each site, the minimum and maximum frequencies are given along with the number of quadrats compared, the weighted mean for the pooled quadrats at the site and the total number of individuals counted at the site. Only quadrats with more than 100 individuals are given. *P*-values are for *G*-tests for within-site heterogeneity in sex ratio. Areas include neighbouring sites separated by $\approx$20 m (Areas 1 and 3) or $\approx$300 m (Areas 5 and 6) of each other

Area	Site	n (quad.)	n (ind.)	Frequency (%)	P
Area 1	PC1	1	436	17.7 (–)	–
	PC2	7	2832	19.5 (6.03–24.1)	<0.001
	PC3	23	8108	12.5 (7.07–22.7)	<0.001
	PC4	11	2477	12.7 (7.24–17.7)	<0.005
	ME*	1	773	5.43 (–)	–
Area 2	CP	2	439	27.3 (23.4–31.3)	NS
Area 3	GR1	6	2271	13.4 (11.8–16.8)	NS
	GR2	15	3657	17.6 (11.7–29.1)	<0.001
Area 4	ML	8	2421	19.0 (10.0–26.0)	<0.001
Area 5	FS†	7	1581	16.6 (10.9–20.0)	NS
	AC	4	573	0.17 (0.00–0.46)	‡
Area 6	AZ	3	1136	24.1 (19.8–31.2)	<0.005
	ES	4	691	0.72 (0.19–1.08)	‡

NS: nonsignificant heterogeneity between quadrats at the site.
*Two samples pooled from the same population, one in 1994, the other in 1995; see Table 2.
†Quadrats either 2.0 × 1.0 m or 3.0 × 1.0 m in area, depending on density.
‡*G*-test not performed, as fewer than 5 males sampled.

same permanent quadrats were compared between years in the field, and when seed was sown and grown up under glasshouse conditions and male frequencies were compared with those of the parents from which the seed had been sampled (Table 2). Thus, when pooled data from the 'parental' generation (first year, sampled in the field) were compared with pooled data from the 'progeny' generation (second year, sampled in the field from the same quadrats or grown in the glasshouse), there were no significant differences in sex ratios. When frequencies were compared across time for individual quadrats, both in the field and with seed grown in the glasshouse, nonsignificant differences predominated, though there were relatively large differences in parental male frequency across time for two quadrats at the ML site (13.4% and 10.5% in the parents vs. 23.7% and 19.2%,

respectively, in the progeny grown in the glasshouse). None of the seeds from entirely monoecious populations, taken from populations south of Marrakech in Morocco, produced any males when sown in the glasshouse.

Sex ratios and stand density

At two sites, density varied substantially between quadrats. At the PC site 1, the frequency of males correlated positively with the log of stand density in quadrats at both the $0.25\,m^2$ scale ($n = 28$, $r = 0.585$, $P < 0.005$) and at the $1.0\,m^2$ scale ($n = 7$, $r = 0.791$, $P < 0.05$; Fig. 1a). This result was repeated at the FS site ($n = 13$, $r = 0.811$, $P < 0.001$; Fig. 1b).

Table 2 Comparisons of male frequencies in quadrats at the ML and the GR sites, and in progeny establishing from seed from those quadrats in the glasshouse and in the same quadrats in the field a year later. All quadrats were $1.0\,m^2$ in area and were contiguous along a transect (in order of their quadrat numbers)

| Quadrat | Field parental generation | | | Progeny generation | | | |
	Males	Cosexes	% males	Males	Cosexes	% males	*P*
ML Site							
1	62	226	21.5	90	388	18.8	NS
2	34	220	13.4	110	354	23.7	<0.001
3	29	260	10.0	68	415	14.1	NS
4	19	162	10.5	92	412	19.2	<0.025
5	46	158	22.5				
6	68	327	17.2				
7	107	305	26.0				
8	94	304	23.6				
Totals	459	1962	19.0	360	1569	18.7	NS
P			<0.001			<0.005	
GR1 Site							
1	34	192	15.0	56	305	15.5	NS
2	40	198	16.8	42	332	11.2	<0.05§
3	36	217	14.2	64	289	18.1	NS
4	35	262	11.8	31	315	8.96	NS
Totals	145	869	14.3	193	1241	13.5	NS
P			NS			<0.001	
GR1 Site*							
1	34	192	15.0	77	543	12.4	NS
4	35	262	11.8	83	554	13.0	NS
Totals	69	454	13.2	160	1097	12.7	NS
P			NS‡				NS
GR2 Site†							
Totals	288	1491	16.2	355	1523	18.9	<0.05§
P			NS‡			<0.005‡	
ME Site†							
Totals	17	371	4.38	25	360	6.49	NS

*Field data from quadrats 1 and 4 sampled in 1994 (parental generation) and from the same quadrats in 1995 ('progeny generation'); other data from field (parents) vs. glasshouse (progeny) comparisons.

†Random quadrats were chosen at the GR2 site in 1994 ($n = 8$) and 1995 ($n = 7$); data are pooled across these random quadrats. *G*-test for significant heterogeneity across quadrats.

‡Total population harvested along a 5 m-long wall at the ME site.

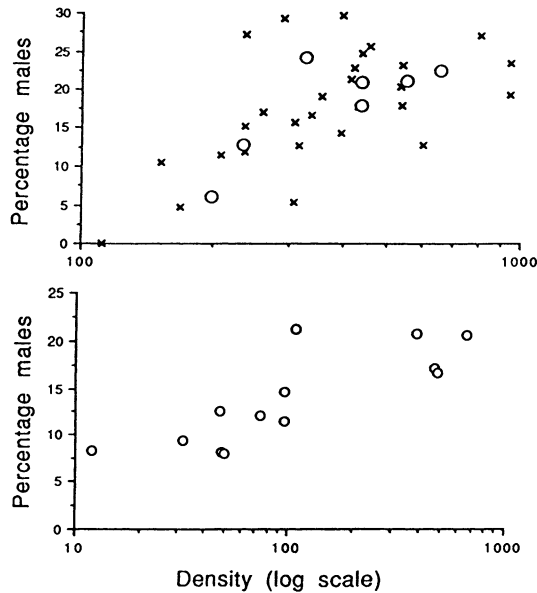§NS after correcting for table-wide error at $P < 0.05$.

Fig. 1 The frequency of males, expressed as a percentage, at (a) PC1 and (b) FS, plotted against stand density. At PC1, quadrats measuring 0.50 × 050 m were laid out along four adjacent quadrats, and data from contiguous quadrats were combined to arrive at data for the seven larger quadrats (1.0 m² squares), shown as open circles. At FS, all quadrats were 1.0 m² in area and were sampled randomly along a 100 m transect. See text for details of correlation analyses.

Sex allocation and quantitative gender

Resource allocation to male and female function in the field was highly variable between plants and quadrats. Both the number of seeds produced by cosexes (seeds on the plant as well as total seed production up to the time of harvest) and the dry weight of staminate flowers produced by cosexes and males varied significantly between quadrats (nonparametric Kruskal–Wallis test: $P < 0.001$ for all attributes tested; Table 3). Males produced a mean of over six times more pollen per biomass than did cosexes and were over 1.5 times as tall (Table 3). Males and cosexes did not differ significantly in their above-ground biomass.

Trade-off in sex allocation

Cosexes also differed widely between quadrats in their allocation of biomass to pollen relative to seeds (Table 3). However, I could find no clear evidence for a direct trade-off in sex allocation between male and female function in cosexes. The log of staminate floral production (mg) was analysed by a GLM for cosexes sampled from 17 quadrats. The effect of plant size was accounted for by including the log of the above-ground biomass as a covariate, and a correlation between male and female allocation was sought in each quadrat by including the log of the number of seeds counted on the plant as a fixed factor nested in quadrats. Male allocation generally correlated positively with seed production after the effect of plant biomass had been accounted for (Table 4a); in eight of the 17 quadrats sampled, the positive regression coefficients relating male to female allocation were significant (Table 4b). At the ES site, about 5% of cosexes were completely male-sterile (producing staminate flowers with empty anthers), but these produced no more seeds than fully male-fertile cosexes ($P > 0.24$).

Possible effect of shading

Cosexes at the GR site under shade produced significantly fewer seeds ($P = 0.033$) and a lower dry fruit weight ($P = 0.014$) per plant biomass than those in the sun, but there was no significant difference between shaded and unshaded quadrats in the pollen productivity of cosexes (Table 5) or in the ratio of pollen produced per seed ($P = 0.221$). In contrast, the production of pollen per unit biomass in males was significantly lower in the shaded quadrats ($P = 0.038$; Table 5). These patterns were further reflected in the contrasting distribution of quantitative gender between shade and sun (Fig. 2): cosexes exposed to full sunlight were quantitatively more female than those growing in deep shade. (Of course, these quad-

Table 3 Variation between quadrats in plant growth and reproductive attributes for males and cosexes in and around Seville. The number of qu: ⁻¹ :ats, *n*, for each comparison is given. Medians were calculated for each quadrat

Attribute	*n*	Median (range)	*P**
Cosexes			
Seeds on plant/Plant biomass (g⁻¹)	22	56.5 (26.1–120.8)	<0.001
Total Seeds/Plant biomass (i.e. including dispersed seeds) (g⁻¹)	22	95.8 (37.73–198.4)	<0.001
Staminate floral biomass/Plant biomass (mg.g⁻¹)	19	18.4 (10.5–41.1)	<0.001
Staminate floral biomass/Seeds on plant (mg)	19	3.23 (1.28–15.1)	<0.001
Staminate floral biomass/Fruit biomass	9	2.44 (1.11–13.9)	<0.001
Plant height/Plant biomass (mm.g⁻¹)	22	775 (242–2018)	<0.001
Males			
Staminate floral biomass/Plant biomass (mg.g⁻¹)	19	116.0 (67.8–201.2)	<0.001
Plant height/Plant biomass (mm.g⁻¹)	18	1491 (398–3300)	<0.001

*Significance of difference between quadrat medians was determined by a Kruskal–Wallis nonparametric test.

Table 4 (a) Anova of the log of staminate floral production (mg) in 17 quadrats sampled at the study sites. 'Biomass' (fixed) was the log of aerial plant biomass (g), and 'Seeds' (fixed, and nested in quadrats) was the log of the number of seeds counted on the plant. (b) Regression results of the general linear model. Quadrats are given in rank order of *P*

(a) ANOVA Source	d.f.	MS	*F*	*P*
Biomass	1	18.191	55.96	<0.001
Quadrat: Q	16	1.414	4.35	<0.001
Seeds (Q)	17	0.872	2.68	<0.001
Error 212		0.325		
Total 246				

(b) Regression Term	Coeff.	SD	*t*-value	*P*
Constant	1.7065	0.5475	3.12	0.002
log (Biomass)	1.1018	0.1473	7.48	<0.001
log (Seeds) (Q)				
1	0.6295	0.1778	3.54	<0.001
2	0.4749	0.1347	3.53	<0.001
3	0.5528	0.1796	3.08	0.002
4	0.6949	0.2242	3.1	0.002
5	0.654	0.2205	2.97	0.003
6	0.5353	0.1942	2.76	0.006
7	0.5918	0.2142	2.76	0.006
8	0.4487	0.1875	2.39	0.018
9	0.533	0.2892	1.84	0.067
10	0.4388	0.3091	1.42	0.157
11	0.2301	0.3172	0.73	0.469
12	0.1538	0.2149	0.72	0.475
13	−0.1623	0.2371	−0.68	0.494
14	−0.1157	0.1901	−0.61	0.543
15	0.1781	0.3029	0.59	0.557
16	0.2338	0.4216	0.55	0.58
17	−0.1435	0.3614	−0.4	0.692

Table 5 Means and standard errors of attributes of sex allocation of cosexes and males in shaded and unshaded quadrats at site GR

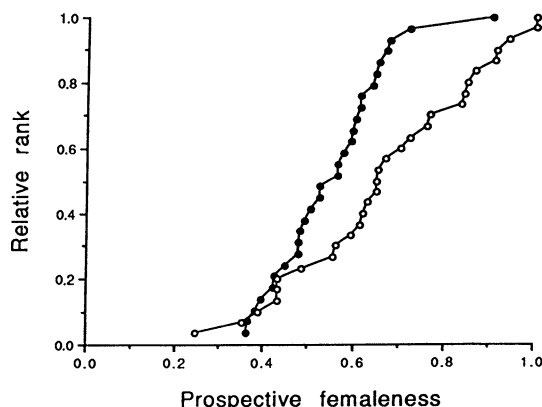| Attribute	Shade	Sun	*P*
Seeds on plant/Plant biomass (g^{-1})	31.6 ± 2.08	59.4 ± 3.84	0.033
Fruit biomass/Plant biomass (mg.g^{-1})	23.2 ± 2.7	81.2 ± 8.9	0.014
Cosex staminate floral biomass/Plant biomass (mg g^{-1})	21.9 ± 3.01	19.4 ± 1.74	NS
Male staminate floral biomass/Plant biomass (mg g^{-1})	93.62 ± 11.6	164.2 ± 23.2	0.038

NS: Non-significant at $P < 0.05$.

Fig. 2 The distribution of prospective femaleness of cosexes in shaded (closed symbols) and unshaded quadrats (open symbols) at the RG site.

rats may have differed in other important ways, and it is by no means certain that shading was the causal factor involved. Nevertheless, the hypothesis seems reasonable, given that biomass production will be strongly light-dependent, and the same patterns were also evident in purely monoecious stands of *M. annua* north of Seville; unpublished data.)

DENSITY EXPERIMENT

Sex ratios

After emergence in the density experiment, there was negligible mortality until plants were harvested 10 weeks after sowing. However, the rate of emergence itself declined steadily with increasing sown density,

from a maximum of 68.8% (at 20 seeds per pot) to a minimum of 39.0% (at 1280 seeds per pot) (Table 6). Male frequency varied between densities in the experiment from 12.9% to 20.4% ($\chi^2 = 15.36$; d.f. = 5; $P = 0.018$; see Table 6), a range consistent with the sex ratios recorded in the field at the PC site where the seed was collected (compare Table 1 with Table 6). However, a χ^2 analysis for trend revealed no directional (linear or logarithmic) trend in the sex ratio with density.

Allocation to pollen and seed production

Cosexes produced less pollen and fewer seeds with increasing density (Fig. 3). After the effect of plant biomass was accounted for, the amount of pollen produced by cosexes at different experimental densities was independent of the resources invested in seed production (analysed as a covariate), i.e. there was no significant correlation or trade-off ($P = 0.34$).

With the (nonsignificant) effect of seed production excluded from the analysis, the six degrees of freedom associated with the (seven levels of the) density treatment were assigned to linear and higher order polynomial components of the log of density, analysed as a fixed, continuous factor. Almost all the variance in staminate floral production between densities (89.0%; $P < 0.001$) could be attributed to the linear component in the model; pollen production by cosexes thus decreased linearly with the log of density. These results point to a significant shift in sex allocation *towards the female function* with increasing density (see Fig. 3c).

Male allocation to floral production, analysed in a similar way to that of cosexes, also decreased nearly linearly with the log of density after the effects of plant size had been accounted for (the linear component of the log of density accounted for 88.0% of the variance associated with density; $P < 0.001$; see Fig. 4).
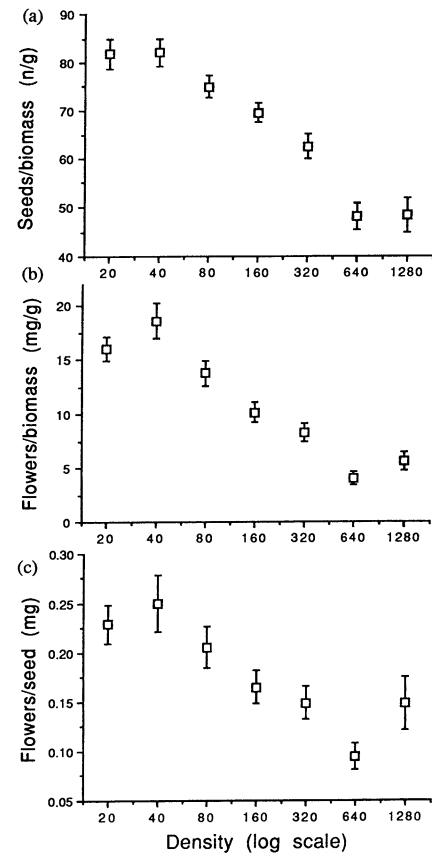

Fig. 3 (a) Seed production and (b) staminate floral production by cosexes as a proportion of above-ground plant biomass, and (c) staminate floral production per seed by cosexes at different densities in the density experiment. Error bars are one standard error ($n = 80$).

Relative male fecundity

The difference in the extent to which males and cosexes reduced their allocation to male function with increasing density meant that the relative male fecundity between males and cosexes differed between densities. Indeed, relative male fecundity, calculated for each density by dividing the mean staminate floral

Table 6 The effect of sown density on the sex expression of established plants in the density experiment. Counts (number sown) were pooled from all pots of each sown density: 32 pots were sown with 20 seeds; 16 pots were sown with 40 seeds; 8 pots were sown with 80 seed¯ and 4 pots were sown with each of 160, 320, 640 and 1280 seeds. The total number of established plants is given as a percentage of the total number of seeds sown at the corresponding density; the total number of immature plants (plants yet to flower) is given as a percentage of established plants; and the total numbers of males and cosexes are given as percentages of sexually mature plants

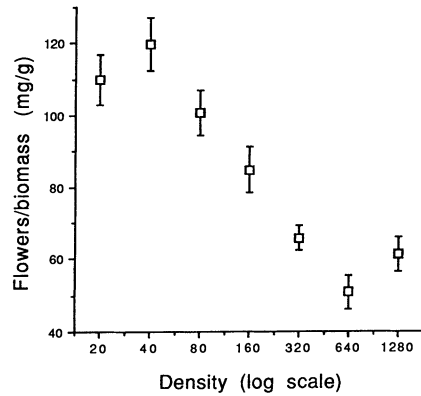| Sown density	Number of seeds	Percentage			
		Established	Immature	Males	Cosexes
20	640	68.8	4.53	12.9	87.1
40	640	59.2	7.50	19.0	81.0
80	640	60.6	11.4	19.0	81.0
160	640	53.3	14.2	16.8	83.2
320	1280	49.6	13.9	20.4	79.6
640	2560	40.9	12.5	13.8	86.2
1280	5120	39.0	10.8	16.8	83.2

Fig. 4 Staminate floral biomass production by males as a proportion of above-ground plant biomass at different densities in the density experiment. Error bars are one standard error ($n = 40$).

biomass per plant biomass for males by that for cosexes, increased significantly with density (Spearman rank correlation, $r = 0.893$; $P = 0.0034$).

Cosexual quantitative gender

The quantitative gender of cosexes ranged almost exclusively between 0.4 and 1.0 in each of the density treatments (Fig. 5). A few plants lay further towards the male end of the range, and six of the plants sampled (four of them in the highest density treatment) were quantitatively male according to the index used here. These 'males' were all small individuals. In fact,
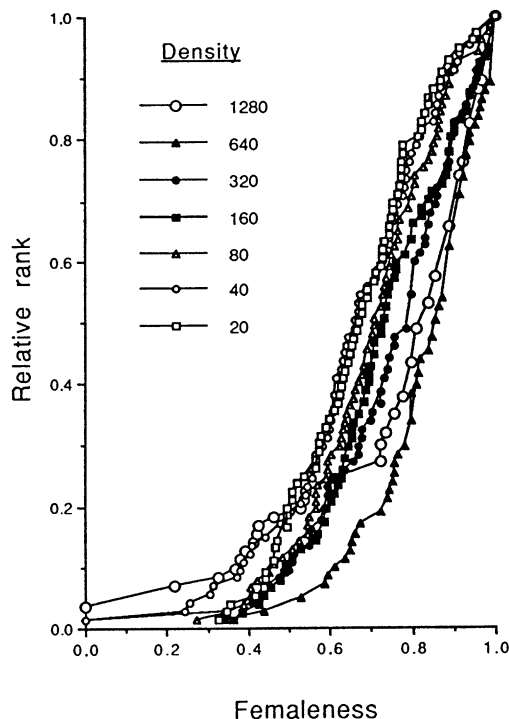

Fig. 5 The prospective femaleness of cosexes sampled from each of the density treatments of the density experiment, plotted in order of its rank in the sample. Plants with a gender value of one are functionally female, while those lying at zero are functionally male. Ranks have been standardized for each density to lie between zero and one.

quantitative gender declined significantly with plant size in the experiment ($P < 0.001$). Figure 5 shows clearly that the femaleness of cosexes increased at higher densities. This trend was significant; after plant size effects were accounted for, quantitative gender increased significantly ($P < 0.001$) with the linear component of density (which accounted for 65.6% of the variance due to density).

Discussion

Table 7 summarizes the patterns of variation in sex ratios and sex allocation found in *Mercurialis annua* in this study.

VARIATION IN MALE FREQUENCIES

Male frequencies in androdioecious populations of *M. annua* were always lower than about 30%, conforming well to theoretical expectations for functional androdioecy (Lloyd 1975; Charlesworth & Charlesworth 1978). However, there was marked spatial heterogeneity in sex ratios which was characterized in this study at three scales: (1) locally between quadrats within uninterrupted stands: sex ratios in adjacent $0.25 \, m^2$ and $1.0 \, m^2$ quadrats were often significantly different; (2) between stands or 'subpopulations': male frequencies differed significantly between isolated or semi-isolated stands separated from one another by five to 50 m; and (3) between distinct populations within the same region, as documented previously by Durand (1963).

Much of this variation is probably the result of genetic differences between populations. The stark differences in male frequency between neighbouring monoecious and androdioecious populations are most likely to have been due to genetic differences in their founders. Pannell (1996) found that maleness in *M. annua* is determined by the presence of a dominant allele at a single nuclear locus, with cosexes homozygous at that locus. This means that androdioecious populations can only be established by the immigration of both males and cosexes.

The strong heterogeneity in male frequencies within populations is particularly interesting. One possible explanation, which would require that both pollen and seed dispersal be highly restricted, is that populations themselves are genetically highly structured. Wind dispersal of pollen tends to be highly leptokurtic and may result in small neighbourhood sizes (Levin & Kerster 1974; Lemen 1980; Farris & Mitton 1984; Tonsor 1985). Seed dispersal is also frequently very local (Levin & Kerster 1974; van Damme 1986). However, it is difficult to account for such small neighbourhood sizes in *M. annua* on the basis of restricted seed dispersal. Seeds are dispersed ballistically from their capsules, and I have observed them falling up to two metres from their parent plant. More importantly, seeds are dispersed secondarily in

Table 7 Summary of salient features of sex ratio and sex allocation variation in *M. annua* in the field and in the glasshouse

Sex ratio variation in the field	
Spatial variation	High at all spatial scales examined
Temporal variation	Lower between generations than between quadrats
Effect of stand density	Increased male frequency at higher densities
	Sex ratio variation in the glasshouse
Progeny sex ratio	Reflects field parental sex ratios
Effect of density	Significant heterogeneity between densities
	Correlation with density nonsignificant
Sex allocation in the field	
Variability	High between plants and between quadrats
Trade-off or correlation?	Allocation to sexual functions correlated in cosexes
Relative male fecundity	High, between about 4 and 10
Secondary sexual attributes	Males taller than cosexes for their mass
	Male inflorescence pedunculate, erect
	Cosexual inflorescence axillary, sessile
Effect of shade	Reduced allocation to reproduction in both sexes
	Greater maleness in cosexes
Sex allocation in density experiment	
Effect of increasing density	Reduced allocation to reproduction in both sexes
	Greater femaleness in cosexes
	Increased relative male fecundity

M. annua by ants (attracted by the lipid- and protein-rich caruncle) which may carry seeds several metres to their nests (Pacini 1990; and personal observation).

The significant correlation between male frequencies and stand density in two of the populations examined implicates an environmental influence on sex expression. This is supported by the significant heterogeneity in sex ratios between densities in the glasshouse experiment. Although this heterogeneity did not constitute a significant correlation between male frequency and density in this study, Pannell (1996) did find a significant correlation in an experiment with lower stand densities which were more closely monitored over time, confirming a causative link between density and sex expression. In that study (Pannell 1996), I showed that at least some genetic males are sexually labile and capable of functioning as cosexes. It appears that labile genetic males are more likely to function as cosexes at lower stand densities. The adaptive significance of this sexual lability is clear, especially given that *M. annua* is a colonizing species whose populations may be founded initially by a very few seeds: Sexually labile genetic males can enjoy the advantage of high outcrossing success in dense, established stands by functioning as males, while they may avoid the risk of reproductive failure during colonization or in sparse stands by becoming cosexual.

VARIATION IN SEX ALLOCATION

A common assumption in models of sex allocation is that the total proportion of resources allocated to reproduction as opposed to vegetative growth and tissue maintenance is fixed, and that this fixed share is partitioned between male and female function (Charnov 1982; Lloyd 1983, 1987b; Brunet 1992). This is the source of the idea that plants which save on allocation to one sex may 'compensate' (Darwin 1877) by re-allocating resources to the other. In *M. annua*, differential pollen productivity between males and cosexes provides strong evidence for such re-allocation of resources, with males producing between five and 10 times as much pollen as cosexes which have to allocate resources to seeds. The fact that evidence for a trade-off between the sexual functions was not found among cosexes (there tended to be a positive, rather than a negative, correlation between male and female functions, even after plant size had been accounted for), simply suggests that variation in resource allocation between sexual functions among cosexes is small relative to variation in the acquisition of resources (due, for example, to genetic differences between individuals, to environmental heterogeneity or to accelerating resource acquisition curves with plant size, as found here; van Noordwijk & de Jong 1986).

THE APPARENT AFFECT OF SHADING

In quadrats shaded by trees, both sexes reduced their allocation to reproduction, as one might expect under a reduced rate of photosynthesis, but whereas males reduced their allocation to pollen production, cosexes only reduced their allocation to female function. The hypothesis that shading rather than other factors, which may also differed between the sites, was responsible for the observed differences in allocation must remain tentative until experimental evidence is found. One possible effect of shading on the optimal sex

allocation in cosexes might be to alter the relative cost of producing pollen and seed. Reduced female allocation by shaded plants might then be explained if it was relatively more costly to produce seeds than pollen under conditions of reduced sunlight. It would also suggest that the *increased* femaleness of cosexes in dense stands (see below) occurs in spite of the greater costs this might involve.

SEX ALLOCATION, DENSITY AND PLANT SIZE

In *M. annua*, while total allocation to reproduction was reduced at higher densities in both males and cosexes, the *relative* allocation of resources to pollen and seed in cosexes also depended on density. At higher densities, cosexes reduced their investment to male function more than they did to female function, with a resulting shift in quantitative gender towards greater femaleness. A similar shift in gender was evident within individual stands in the field, where smaller plants also tended to greater femaleness. The latter observation should be expected for a wind-pollinated plant, in which pollen dispersed by small plants growing up under larger ones is likely to be relatively unsuccessful (Niklas 1985; Bickel & Freeman 1994; de Jong & Klinkhamer 1994). But what outcrossing advantages might accrue to cosexes in dense stands with greater relative female function?

There are several possibilities. One is that local mate competition causes male fitness gains to saturate (Lloyd & Bawa 1984). However, such an explanation is implausible for a wind-pollinated species, in which outcrossing success is much more likely to increase with stand density, as noted above. Moreover, local mate competition could not select for both an adaptive increase in male frequency and a decrease in cosex allocation to male function. Instead, I suggest that cosexuality has not evolved in *M. annua* as a result of selection for outcrossing at all, but for the ability to self-fertilize under conditions where selfing would be an advantage.

The clearest support for this hypothesis comes from the fact that the pollen produced in the staminate flowers of the cosexual inflorescence, which closely surround a pistillate flower, is well positioned for potential self-fertilization, whereas it rates poorly in its prospects for outcrossing when compared with the pollen produced in flowers in the male inflorescence, held on erect peduncles and extended above the plant (Pannell 1995, 1997; see Niklas 1985). It is well known that partially self-fertilizing species spend less on producing pollen than outcrossing species (e.g. Lloyd 1987a), and this appears very much to be so in *M. annua*, where cosexes produce between about 1/10 and 1/4 as much pollen as males (see below).

Why should the capacity for self-fertilization have evolved in *M. annua*, a species whose diploid populations and congeneric relatives are all fully dioecious? A plausible explanation is that cosexuality has evolved

in *M. annua* as a result of the greater prospective colonization success in self-fertile cosexes relative to unisexuals (Pannell 1997b). This selective advantage of 'reproductive assurance' has long been seen as responsible for the common association found between a colonizing ability and self-fertilization (Baker 1955, 1967; Jarne & Charlesworth 1993; Pannell 1997b). It is particularly significant that in *M. annua*, dioecy occurs throughout central and western Europe where large, dense populations often persist from one year to the next, while cosexuality is found in the drier western Mediterranean region where the species is a winter annual with a short growing season. Androdioecious populations occur in a relatively restricted zone where populations are particularly abundant and large, where conditions thus appear to give rise to greater outcrossing rates, and where the selective pressure for reproductive assurance is likely to be reduced.

THE MAINTENANCE OF ANDRODIOECY IN *M. ANNUA*

The extreme rarity of androdioecy has commonly been attributed to the perceived unlikelihood of males arising with more than twice the male fertility of cosexes. This is because it is implicitly assumed that cosexes are equisexual (*sensu* Lloyd 1980), i.e. that they invest half of their reproductive resources to their male and half to their female function. Such would imply that even complete compensation for a loss of female function in males would result in only a twofold increase in their male fertility, at best. But if cosexes allocate a proportion $1/r$ of their reproductive resources to male function, complete re-allocation of resources from female to male function would result in an r-fold increase in male fertility. Thus, if cosexes spent less than $1/2$ of their resources on male function, males would be more than twice as fertile as the male function of the cosexes, and they could spread. This has not been appreciated in other accounts of the evolution of androdioecy (e.g. Lloyd 1975; Charlesworth & Charlesworth 1978; Charlesworth 1984; Fritsch & Rieseberg 1992), and it is almost certainly what has allowed males to spread in *M. annua*.

Males in *M. annua* produce between 4 and 10 times the staminate floral biomass of cosexes, which would suggest, if we conservatively assume complete re-allocation of resources, that cosexes allocate between 1/4 and 1/10 of their reproductive resources to male function (less than complete re-allocation would imply an even greater bias to female function in cosexes). When selfing rates are sufficiently high (e.g. when stand densities are low), males with high relative pollen production and dispersal may not be able invade a cosexual population (Charlesworth 1984). In a dense wind-pollinated stand, however, the outcrossing rate will be increased, and the same males might easily invade and spread. *M. annua* males are unlikely to be suc-

cessful in very sparse stands (e.g. soon after colonization), but in dense stands occupied by cosexes with female biased sex allocation, androdioecy may readily evolve. In such a situation, cosexes will be selected to shift their sex allocation further towards their female function, with the potential evolution of dioecy from androdioecy (Pannell 1995).

I suggest that androdioecy persists in *M. annua* because of continued selective pressure for reproductive assurance, maintaining both female-biased cosexuality (because populations must be founded initially) as well as males with pedunculate inflorescences (because such males are highly successful mates in dense stands once they are established). The fact that some genetic males also possess an ability to function as cosexes in sparse stands (Pannell 1996; see above) almost certainly contributes to the maintenance of androdioecy in the species.

Acknowledgements

References

Ashman, T. (1994) Reproductive allocation in hermaphroditic and female plants of *Sidalcea oregana* spp. *spicata* (Malvaceae) using four currencies. *American Journal of Botany*, **81**, 433–438.

Baker, H.G. (1955) Self-compatibility and establishment after 'long-distance' dispersal. *Evolution*, **9**, 347–348.

Baker, H.G. (1967) Support for Baker's Law – as a rule. *Evolution*, **21**, 853–856.

Barrett, S.C.H. (1992) Gender variation and the evolution of dioecy in *Wurmbea dioica* (Liliaceae). *Journal of Evolutionary Biology*, **5**, 423–444.

Bawa, K.S. (1980) Evolution of dioecy in flowering plants. *Annual Review of Ecology and Systematics*, **11**, 15–39.

Bawa, K.S. & Beach, J.H. (1981) Evolution of sexual systems in flowering plants. *Annals of the Missouri Botanic Garden*, **68**, 254–274.

Bickel, A.M. & Freeman, D.C. (1994) Effects of pollen vector and plant geometry on floral sex ratio in monoecious plants. *American Midland Naturalist*, **130**, 239–247.

Brunet, J. (1992) Sex allocation in hermaphroditic plants. *Trends in Ecology and Evolution*, **7**, 79–84.

Bulmer, M. (1986) Sex ratios in geographically structured populations. *Trends in Ecology and Evolution*, **1**, 35–38.

Charlesworth, D. (1984) Androdioecy and the evolution of dioecy. *Biological Journal of the Linnean Society*, **22**, 333–348.

Charlesworth, D. & Charlesworth, B. (1978) A model for the evolution of dioecy and gynodioecy. *American Naturalist*, **112**, 975–997.

Charlesworth, D. & Charlesworth, B. (1981) Allocation of resources to male and female functions in hermaphrodites. *Biological Journal of the Linnean Society*, **15**, 57–74.

Charnov, E.L. (1982) *The Theory of Sex Allocation*. Princeton University Press, Princeton, NJ.

Charnov, E.L., Maynard Smith, J. & Bull, J.J. (1976) Why be an hermaphrodite? *Nature*, **263**, 125–126.

van Damme, J.M.M. (1986) Gynodioecy in *Plantago lanceolata* L. IV. Frequencies and spatial distribution of nuclear and cytoplasmic genes. *Heredity*, **56**, 355–364.

van Damme, J.M.M. & van Delden, W. (1984) Gynodioecy in *Plantago lanceolata* L. IV. Fitness components of sex types in different life cycle stages. *Evolution*, **38**, 1326–1336.

Darwin, C. (1877) *The Different Forms of Flowers on Plants of the Same Species*. Appleton, New York.

Donoghue, M.J. (1989) Phylogenies and the analysis of evolutionary sequences, with examples from seed plants. *Evolution*, **43**, 1137–1156.

Durand, B. (1963) Le complexe *Mercurialis annua* L. *s.l.*: une étude biosystematique. *Annales des Sciences Naturelles, Botanique, Paris*, **12**, 579–736.

Durand, R. & Durand, B. (1992) Dioecy, monoecy, polyploidy and speciation in annual Mercuries. *Bulletin De La Societé Botanique De France Lettres Botaniques*, **139**, 377–399.

Farris, M.A. & Mitton, J.W. (1984) Population density, outcrossing rate, and heterozygous superiority in Ponderosa pine. *Evolution*, **38**, 1151–1154.

Frank, S.A. (1989) The evolutionary dynamics of cytoplasmic male sterility. *American Naturalist*, **133**, 345–376.

Fritsch, P. & Rieseberg, L.H. (1992) High outcrossing rates maintain male and hermaphrodite individuals in populations of the flowering plant *Datisca glomerata*. *Nature*, **359**, 633–636.

Givnish, T.J. (1980) Ecological constraints on the evolution of breeding systems in seed plants: dioecy and dispersal in Gymnosperms. *Evolution*, **34**, 959–972.

Hamilton, W.D. (1967) Extraordinary sex ratios. *Science*, **156**, 477–488.

Jarne, P. & Charlesworth, D. (1993) The evolution of the selfing rate in functionally hermaphrodite plants and animals. *Annual Review of Ecology and Systematics*, **24**, 441–466.

de Jong, T.J. & Klinkhamer, G.L. (1994) Plant size and reproductive success through female and male function. *Journal of Ecology*, **82**, 399–402.

Lemen, C. (1980) Allocation of reproductive effort to the male and female strategies in wind-pollinated plants. *Oecologia*, **45**, 156–159.

Lepart, J. & Dommee, B. (1992) Is *Phillyrea angustifolia* L. (Oleaceae) an androdioecious species? *Botanical Journal of the Linnean Society*, **108**, 375–387.

Levin, D.A. & Kerster, H.W. (1974) Gene flow in seed plants. *Evolutionary Biology*, **7**, 139–220.

Liston, A., Rieseberg, L.H. & Elias, T.S. (1990) Functional androdioecy in the flowering plant *Datisca glomerata*. *Nature*, **343**, 641–642.

Lloyd, D. (1975) The maintenance of gynodioecy and androdioecy in angiosperms. *Genetica*, **45**, 325–339.

Lloyd, D.G. (1979) Parental strategies in angiosperms. *New Zealand Journal of Botany*, **17**, 595–606.

Lloyd, D.G. (1980) Sexual strategies in plants. III. A quan-

69

John Pannell

titative method for describing the gender of plants. *New Zealand Journal of Botany*, **18**, 103–108.

Lloyd, D.G. (1983) Evolutionary stable sex ratios and sex allocations. *Journal of Theoretical Biology*, **105**, 525–539.

Lloyd, D.G. (1984) Gender allocation in outcrossing cosexual plants. *Principles of Plant Population Ecology* (eds R. Dirzo & J. Sarukhan), pp. 277–300. Sinauer, Sunderland, MA.

Lloyd, D.G. (1987a) Allocations to pollen, seeds and pollination mechanisms in self-fertilizing plants. *Functional Ecology*, **1**, 83–89.

Lloyd, D.G. (1987b) Parallels between sexual strategies and other allocation strategies. *The Evolution of Sex and its Consequences* (ed. S. C. Stearns), pp. 263–281. Birkhauser Verlag, Basel.

Lloyd, D.G. & Bawa, K.S. (1984) Modification of the gender of seed plants in varying conditions. *Evolutionary Biology*, **17**, 255–336.

Niklas, K.J. (1985) The aerodynamics of wind-pollination. *Botanical Review*, **51**, 328–386.

van Noordwijk, A.J. & de Jong, G. (1986) Acquisition and allocation of resources: their influence on variation in life history tactics. *American Naturalist*, **128**, 137–142.

Pacini, E. (1990) *Mercurialis annua* L. (Euphorbiaceae) seed interactions with the ant *Messor structor* (Latr.). Hymenoptera. Formicidae. *Acta Botanica Neerlandica*, **39**, 253–262.

Pannell, J.R. (1995) *Models of androdioecy and studies on* Mercurialis annua *L*. DPhil thesis, University of Oxford.

Pannell, J.R. (1996) Mixed genetic and environmental sex determination in an androdioecious population of *Mercurialis annua. Heredity*, in press.

Pannell, J.R. (1997a) Widespread functional androdioecy in *Mercurialis annua* L. (Euphorbiaceae). *Biological Journal of the Linnean Society*, in press.

Pannell, J.R. (1997b) The maintenance of gynodioecy and androdioecy in a metapopulation. *Evolution*, in press.

Philbrick, C.T. & Rieseberg, L.H. (1994) Pollen production in the androdioecious *Datisca glomerata* (Datiscaceae): Implications for breeding system equilibrium. *Plant Species Biology*, **9**, 43–46.

Thomson, J.D. & Brunet, J. (1990) Hypotheses for the evolution of dioecy in seed plants. *Trends in Ecology and Evolution*, **5**, 11–16.

Tonsor, S.J. (1985) Leptokurtic pollen-flow, non leptokurtic gene-flow in a wind-pollinated herb, *Plantago lanceolata* L. *Oecologia*, **67**, 442–446.

Traveset, A. (1994) Reproductive biology of *Phillyrea angustifolia* L. (Oleaceae) and effect of galling-insects on its reproductive output. *Botanical Journal of the Linnean Society*, **114**, 153–166.

Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M. & Webb, D.A. (1968) *Flora Europaea*, Vol. 2. Cambridge University Press, Cambridge.

Valdes, B., Talavera, S. & Fernandez-Galiano, E. (1987) *Flora Vascular de Andalucia Occidental*, Vol. 2. Ketres Editora, Barcelona.

Willson, M.F. (1979) Sexual selection in plants. *American Naturalist*, **113**, 777–790.

Willson, M.F. (1990) Sexual selection in plants and animals. *Trends in Ecology and Evolution*, **5**, 210–214.