SEX EXPRESSION AND SEXUAL DIMORPHISM IN SUBDIOECIOUS
WURMBEA DIOICA (COLCHICACEAE)

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Subdioecy is a sexual system with male, female, and hermaphrodite phenotypes and is often considered a transitory stage in the evolution of full dioecy. Here, we examine sex ratios, sex expression, and sexual dimorphism in three subdioecious populations of *Wurmbea dioica* to gain insight into the stability of this sexual system. Sex ratios in the field were slightly male biased: 50% males, 43% females, and 7% hermaphrodites. Sex expression of females was constant for 3 yr under favorable glasshouse conditions. Male sex expression was labile, and 71% of males became hermaphrodites. Hermaphrodites remained unchanged, probably in response to the favorable conditions. In the field, estimates of femaleness of hermaphrodites tended toward maleness ($G = 0.31$). Females produced three times more seeds per plant than did hermaphrodites. This increased fecundity resulted from females producing more ovuliferous flowers per plant and more ovules per flower but fewer aborted seeds (21% vs. 51%). Hermaphrodites were self-compatible, and their lower seed set may reflect inbreeding depression and/or differences in allocation patterns compared with females. Males produced 1.3 times more pollen per flower that was 7% more viable than did hermaphrodites, indicating resource-based trade-offs between male and female functions when males produced functional pistils. Males produced flowers that were slightly larger in diameter than those of hermaphrodites, and flowers of both were 1.3 times larger than those of females. Males and hermaphrodites produced similar numbers of flowers, both producing more than females. Fecundity differences between females and hermaphrodites are consistent with models for the maintenance of constant females. Male sex lability may be advantageous if male fitness gain curves decelerate in response to increased resource availability and seed production augments plant fitness. This is consistent with resource-based threshold models of male gender modification.

Keywords: gender modification, sex expression, sex lability, sexual dimorphism, subdioecy, *Wurmbea dioica*.

Introduction

One common evolutionary pathway from hermaphroditism to dioecy is via gynodioecy and subdioecy (Charlesworth and Charlesworth 1978; Ross 1982; Webb 1999). The first step of this pathway is the introduction and establishment of male-sterile (females) mutants into a population of hermaphrodite individuals followed by the establishment of female-sterile (males) mutants. Selective forces promoting the evolution of dioecy via this pathway have been studied predominantly from two perspectives, resource compensation and inbreeding avoidance (Lloyd 1975; Charlesworth and Charlesworth 1978; Thomson and Brunet 1990; Webb 1999). Resource compensation models assume trade-offs between male and female functions and assume that reproductive success can be increased by specializing in one sexual function (Charnov 1982; Geber 1999). Inbreeding avoidance models assume that females are obligate outcrossers, whereas hermaphrodites are self-compatible, and their selfed progeny exhibit inbreeding depression (Charlesworth and Charlesworth 1978; Charles-

worth 1999). These models are not mutually exclusive, and both resource compensation and inbreeding avoidance are probably important in providing the necessary advantage for females and males to establish in a hermaphrodite population (Thomson and Brunet 1990; Charlesworth 1999).

Subdioecious populations contain male, female, and hermaphrodite phenotypes with males often exhibiting sex lability (i.e., fruiting or inconstant males; Lloyd 1976; Lloyd and Bawa 1984; Delph and Lloyd 1991; Sakai and Weller 1991; Barrett et al. 1999). Subdioecy is often considered a transitory stage in the evolution of full dioecy (Charlesworth and Charlesworth 1978; Ross 1982; Webb 1999). The shift to full dioecy may occur under stressful ecological conditions if hermaphrodites cannot maintain both sexual functions successfully or if inbreeding depression in hermaphrodites is intensified (Sakai and Weller 1991; Delph and Lloyd 1996; Charlesworth 1999). Under less stressful and heterogeneous conditions, however, subdioecy might be stable if males increase their fitness by occasionally producing seeds when resources are abundant. Such fitness increases would require linear or accelerating male gain curves under normal conditions but decelerating (i.e., saturating) gain curves under conditions of abundant resources. This model assumes that pollen production, pollen export, plant survival, or future reproduction are not adversely affected by the production of ovules and seeds (Seger and Eck-

hart 1996). To provide further insight into the stability of subdioecy, assessment of the ecological conditions influencing sex lability as well as comparisons of resource allocation patterns among the sexual phenotypes and the likelihood of inbreeding depression are required.

Wurmbea dioica (Colchicaceae) exhibits pronounced intraspecific variation in sexual systems that range from cosexual to dioecious populations through varying degrees of gynodioecy and subdioecy (Macfarlane 1980; Ornduff 1985; Barrett 1992). Following an extensive survey of populations in southern Australia, Barrett (1992) found that full dioecy was associated with arid conditions, whereas subdioecy was associated with benign conditions, and suggested that dioecy evolved from hermaphroditism via gynodioecy. In southeastern Australia, populations are often subdioecious, containing male, female, and hermaphrodite phenotypes that exhibit sexual dimorphism (Barrett 1992; Vaughton and Ramsey 1998; Barrett et al. 1999). Hermaphrodites generally comprise less than 10% of plants, although under favorable environmental conditions frequencies in some populations can reach ca. 30% (Barrett 1992; M. Ramsey, unpublished data). In a field demographic study, Barrett et al. (1999) found that female plants exhibited canalized gender, producing only ovuliferous flowers, whereas some male plants were sexually labile, producing varying numbers of polliniferous and perfect flowers (i.e., fruiting males). Gender modification in male plants was size dependent with increased size associated with the production of perfect flowers (Barrett et al. 1999).

Here, we examine sex expression and sexual dimorphism in three subdioecious populations of *W. dioica* ssp. *dioica* in southeastern Australia, augmenting previous work of Barrett et al. (1999). To investigate sex expression in these populations, we examine sex ratios; assess sex lability of males, females, and hermaphrodites under controlled conditions; and quantify functional gender. To investigate sexual dimorphism, we examine resource allocation patterns by comparing reproductive and vegetative traits among phenotypes. Because the relative fertilities of the sexual phenotypes determine the stability of subdioecy, we were particularly interested in comparing ovule and seed production between females and hermaphrodites and pollen production between males and hermaphrodites. Finally, we assess the degree of self-compatibility of hermaphrodites and compare open-pollinated seed set and seed abortion of hermaphrodites and females to assess the likelihood of inbreeding depression.

Material and Methods

Study Species and Sites

Wurmbea dioica (R. Br.) F. Muell. is a small perennial geophyte that is widely distributed throughout southern Australia (Macfarlane 1980). Populations in southwestern Australia are monomorphic or dimorphic in sex expression (*W. dioica* ssp. *alba*), whereas populations in southeastern Australia are sexually dimorphic and often subdioecious (*W. dioica* ssp. *dioica*; Barrett 1992). Flowering plants of *W. dioica* ssp. *dioica* have an underground corm, two to three annual leaves, and an inflorescence spike with one to eight flowers. Plants produce either all ovuliferous (female), all polliniferous (male), or vary-

ing proportions of polliniferous and perfect (hermaphrodite) flowers. Leaves emerge in winter, and flowering occurs in spring (August–October). Flowers are insect pollinated and have six white, elliptic tepals (4–11 mm long) in a single whorl. Each tepal has a purple nectary located toward the base. Fruits are capsules containing 10–50 seeds (Macfarlane 1980; Barrett 1992; Vaughton and Ramsey 1998).

We conducted this study in 1996 at three open woodland sites (Yan Yean [YY], Whittlesea [WH], and Wallan [WA]), ca. 50 km north of Melbourne, in southern Victoria (37°34'S, 145°06'E). YY and WH were ca. 10 km apart, and both were ca. 25 km from WA. All sites experience cool, wet winters and hot, dry summers. The populations that we studied were ca. 600 km south of those studied by Barrett et al. (1999) in the Australian Capital Territory.

Sex Ratio and Sex Lability

We scored the sex of 112–159 plants in each population on three different occasions, each ca. 2 wk apart and encompassing peak flowering (total $n = 1571$ plants). We conducted the first census ca. 4 wk after flowering had commenced and, thus, did not collect data on field sex ratios at the beginning of the flowering season. Plants were scored while walking transects through the populations. We used a multinomial logit model and analysis of deviance assuming a Poisson error distribution to examine the effects of census date and population as explanatory variables on the number of plants of each sex, a response variable (Crawley 1993).

To assess sex lability, in 1996 we excavated male ($n = 30$), female ($n = 30$), and hermaphrodite ($n = 23$) flowering plants from the YY population, transplanted them into a mixture of sand and peat (3 : 1) in 10-cm pots, and placed them in a shade house. Plants were watered regularly and fertilized bimonthly with 100 mL of half-strength water soluble fertilizer (Aquasol; Hortico [Aust], Laverton North, Victoria, Australia). Sexual phenotype of flowering plants was recorded over 3 yr.

Ovule, Seed, and Pollen Production

To examine the number of ovules produced by females and hermaphrodites, we collected the first flower on inflorescences of 20 plants of each sex in the three populations. Ovaries were dissected, and ovules were counted using a dissecting microscope. We examined the number of open-pollinated seeds produced by 20–25 females and hermaphrodites in each population. Plants were marked, and the number of ovuliferous flowers per plant was noted. Ca. 6–8 wk later, the fruits were harvested, and the number of seeds per plant was counted. We examined percentage seed set of the first flower to compare the conversion of ovules to seeds independently of the number of ovules per flower. Seed set was determined as the percentage of ovules producing seeds. We also examined the percentage of aborted seeds as the number of aborted seeds divided by aborted + filled seeds. Aborted seeds were shriveled and 25%–75% the size of filled seeds. Abortions were probably underestimated because embryos that aborted early in development could not be detected. To assess the effects of sex and population on ovule production, percentage seed set, and percentage seed abortion, we used two-way ANOVAs. For num-

ber of seeds per plant, we assessed the effects of sex and population using ANCOVA, with the number of ovuliferous flowers as a covariate.

To compare seed mass, we weighed 15 seeds individually from 10 females and hermaphrodites in each population. Seeds were collected in November 1996, stored in envelopes, and weighed in February 1997. We assessed the effects of sex and population and plants nested within populations on seed mass using a partially hierarchical ANOVA.

We assessed pollen production from the first flower on inflorescences of 20 male and 20 hermaphrodite plants in each population. Anthers from each flower were collected into 1.5-mL centrifuge tubes. As anthers dehisced, we added 0.5 mL of lactophenol–aniline blue stain and counted the number of pollen grains in four replicate hemocytometer grids. We also estimated the proportion of viable pollen; viable grains stain dark blue, and inviable grains stain pale blue and are misshapen. Some inviable grains may be indistinguishable from normal grains, and this technique may overestimate pollen viability. We assessed the effects of sex and population on pollen production and viability using two-way ANOVAs.

Sexual Dimorphism

In each population, we assessed the length of the second leaf, inflorescence height to the first flower, diameter of the first flower, and the number of flowers on 20 plants of each sex. The second leaf was measured because the first leaf was often damaged; lengths of the second and first leaves are highly correlated ($r = 0.93$, $P < 0.001$, $n = 61$; M. Ramsey, unpublished data). Flowers with fully expanded tepals were measured. Flower diameter was measured as the distance between the tips of two opposite tepals (mean of two measurements). On females and hermaphrodites, we also measured the diameter of the ovary on the first flower (mean of two measurements). Traits were measured to the nearest 0.1 mm with vernier calipers. We used MANOVA to assess the effects of sex and population on all traits simultaneously, omitting ovary diameter from the analysis because males have nonfunctional ovaries. Sex, population, and their interaction were significant (sex: $F_{8,2} = 23.13$, $P = 0.042$; population: $F_{8,336} = 33.87$, $P < 0.001$; sex population: $F_{16,513} = 1.94$, $P = 0.016$), and we, subsequently, examined all traits individually using two-way ANOVAs.

We also compared flower production by counting the number of flowers on 130 plants of each sex in each population. All flowers on hermaphrodites produce pollen but not all produce a functional ovary, and we counted both flower types on these plants. Ovuliferous flowers are clearly distinguished by ovary size, which is greatly reduced in flowers that are polliniferous only. We used two-way ANOVAs to assess the effects of population and sex on flower production. Total flower production was compared among the three sexes, and ovuliferous flower production was compared between females and hermaphrodites.

We measured tepal and nectary areas on the first flower of 11 plants of each sex in each population. Areas were measured from three tepals from each flower with a calibrated eyepiece graticule. Tepal area was calculated using the formula for an ellipse, $A = \pi ab$, where a and b are the radii along the principal

and secondary axes, respectively. Nectary area was calculated as the product of nectary length and width. We used partially hierarchical ANOVAs with plants nested within populations to assess the effects of sex and population on tepal and nectary areas.

Gender Estimates

We quantified gender of hermaphrodites in each population by calculating Gi, the proportion of genes that are likely to be transmitted through ovules (functional femaleness; Lloyd 1980). Here, $Gi = di/(di + liE)$, where di and li are the number of seeds and pollen grains, respectively, produced by plant i. The equivalence factor, $E = \Sigma di/\Sigma li$, measures the ratio of seeds to pollen grains in a population, scaling pollen and seed fitnesses so that both are equal because both parents contribute equal numbers of nuclear genes to seeds. The model assumes that all pollen and all seeds have equal probability of contributing genes to the next generation. Because males produce no ovules and females produce no pollen, $G = 0.0$ for males and $G = 1.0$ for females. Hermaphrodites produce both pollen and ovules, and G can vary between 0.0 and 1.0.

We scored flower number and sex for 495 plants at YY (41 hermaphrodites), 561 plants at WH (31 hermaphrodites), and 515 plants at WA (33 hermaphrodites). For males and hermaphrodites, we estimated pollen production as the product of the number of flowers per plant and the mean number of viable pollen grains per flower, calculated from 20 plants of each sex in each population. We estimated seed production of females as the product of the number of flowers per plant and the mean number of seeds per flower, calculated from 20 plants in each population. We counted all of the seeds produced by each hermaphrodite on the transects. We plotted frequency distributions of the ranks of the individual values and compared G estimates among populations with a one-way ANOVA.

Self-Compatibility of Hermaphrodites

From each population, we excavated seven hermaphrodite plants as the first flower was just opening and transplanted them into an unheated shade house. We self-pollinated the first flower and bagged plants with fine mesh to exclude insect visitors. Fruits were harvested 6–8 wk later, and percentage seed set was determined as the ratio of filled seeds to ovules + filled seeds. We compared percentage self seed set among the populations with a one-way ANOVA.

Statistical Analyses

Measurements, areas, and number of ovules and flowers were transformed using natural logarithms. Other counts were square root transformed, and percentages and gender estimates (Gi) were arcsine transformed. Transformation improved normality and homoscedasticity as established by Shapiro-Wilks's and Levene's tests, respectively. For ANOVAs, sex and population were fixed and random factors, respectively, and plant, when included, was a random factor. If sex was significant in factorial ANOVAs, we then examined differences among sexes with Tukey tests. Means ($\pm$ SE) are given.

Table 1

Effects of Sex and Population on Ovule, Seed, and Pollen Production

Source	Number of ovules			Number of seeds			Percentage seed set			Percentage seed abortion			Number of pollen grains			Percentage pollen viability		
	df	MS	F	df	MS	F	df	MS	F	df	MS	F	df	MS	F	df	MS	F
Sex	1	2.0	20.2*	1	347.9	152.1**	1	7484.5	59.2*	1	12,084	88.8**	1	41,990	115.8**	1	960.2	59.2*
Population	2	0.1	1.3 ns	2	0.3	0.2 ns	2	87.4	0.6 ns	2	164.9	1.3 ns	2	8953	4.0*	2	294.0	5.0*
Sex × population	2	0.1	1.3 ns	2	2.3	0.7 ns	2	126.4	0.8 ns	2	136.1	1.08 ns	2	363	0.2 ns	2	16.2	0.3 ns
Error	114	0.1	...	113	3.2	...	114	153.5	...	114	126.2	...	114	2232	...	114	58.5	...

Note. Ovule and seed production of females and hermaphrodites and pollen production of males and hermaphrodites were compared. Ovule and pollen production and percentage seed abortion and seed set were assessed from the first flower on plants and were analyzed with model 3 ANOVAs. The number of seeds per plant was analyzed with a model 3 ANCOVA, with the number of ovuliferous flowers as a covariate, which was significant ($F_{1,113} = 14.7, P < 0.001$). Sex and populations were fixed and random factors, respectively. Analyses correspond to data in figure 1. ns = not significant.

* $P < 0.05$.
** $P < 0.01$.

Results

Sex Ratio and Lability

Field sex ratio was independent of census date and population and their interaction (date: $\chi^2 = 3.98$, df = 4, $P = 0.41$; population: $\chi^2 = 4.26$, df = 4, $P = 0.37$; date population: $\chi^2 = 3.50$, df = 8, $P = 0.90$). Sex ratios were slightly male biased. Pooled over census dates and populations, sex ratios were: males ≈ 0.50; females ≈ 0.43; and hermaphrodites ≈ 0.07.

Over the 3 yr that sex lability was assessed, 21 male, 17 female, and 16 hermaphrodite plants flowered. Not all plants flowered in all years. Male sex expression was labile, and 15 plants (71.4%) flowered as hermaphrodites in one or more years and six plants flowered as males. Female sex expression was constant; all plants flowered as females. Hermaphrodite sex expression remained unchanged; all plants flowered again as hermaphrodites.

Ovule, Seed, and Pollen Production

Ovuliferous flowers from females produced 1.2–1.4 times more ovules than perfect flowers from hermaphrodites (table 1; fig. 1). All ovuliferous flowers on female and hermaphrodite plants produced fruits. For seeds per plant, the number of ovuliferous flowers, the covariate, was significant (table 1), and positively related to seed production. Females produced 2.5–3.7 times more seeds and percentage seed set was 1.8–2.5 times greater than did hermaphrodites (table 1; fig. 1). In contrast to seed production, seed abortion was significantly greater in hermaphrodites than in females. Abortion in females was only 0.36–0.51 times that of hermaphrodites (table 1; fig. 1). For ovules, seeds, and aborted seeds, populations did not differ and the sex population interactions were not significant (table 1).

Individual seed masses (mg) from females and hermaphrodites were: YY, 0.67 ± 0.02 vs. 0.71 ± 0.02; WH, 0.78 ± 0.02 vs. 0.83 ± 0.02; and WA, 0.84 ± 0.02 vs. 0.84 ± 0.02, respectively. Seed mass did not differ significantly between females and hermaphrodites ($F_{1,2} = 5.30$, $P = 0.148$), although there was a slight trend for hermaphrodites to produce heavier seeds at YY and WH. Seed mass differed among populations ($F_{2,27} = 4.07$, $P = 0.029$) and was greatest at WA and least at YY. The sex population interaction was not significant

($F_{2,27} = 0.09$, $P = 0.919$). Seed mass of plants within populations differed significantly ($F_{27,840} = 16.02$, $P < 0.001$). The sex plants within populations interaction was significant ($F_{27,840} = 11.66$, $P < 0.001$).

Flowers on males produced significantly more pollen grains that had greater viability than did flowers on hermaphrodites (table 1; fig. 1). Total pollen production and viability of males were 1.2–1.4 times and 1.04–1.11 times greater than hermaphrodites, respectively. For pollen production and viability, populations varied significantly, but the sex population inter-
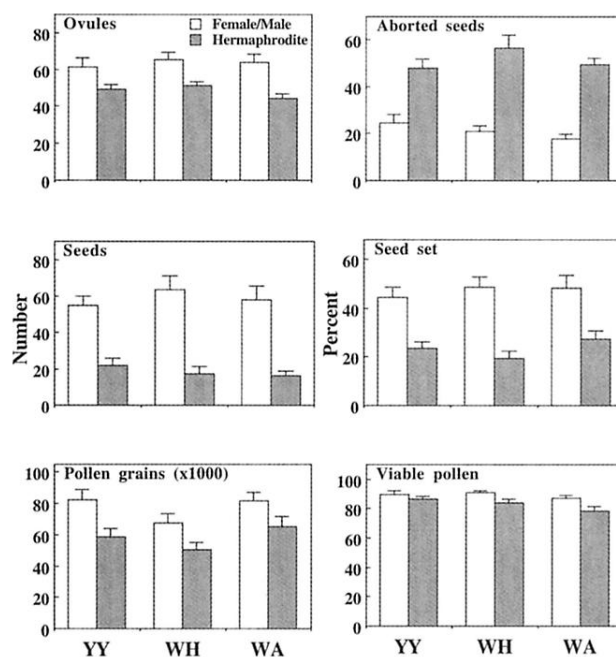

Fig. 1 Mean ($\pm$ SE) number of ovules, seeds, and pollen grains and percentage seed abortion, seed set, and viable pollen of female, hermaphrodite, and male plants ($n = 20$) in three populations of *Wurmbea dioica* (YY, WH, WA). Seed number was counted from all open-pollinated flowers on plants. Other traits were assessed from the first flower. Females produced more ovules and seeds and converted more ovules into seeds but had fewer aborted seeds than hermaphrodites (all $P < 0.05$). Males produced more pollen of higher viability than hermaphrodites (both $P < 0.05$). Analyses are given in table 1.

Table 2

Effects of Sex and Population on Plant Traits of Males, Females, and Hermaphrodites

Source	Leaf length			Inflorescence height			Flower diameter			Flower number			Ovary diameter		
	df	MS	F	df	MS	F	df	MS	F	df	MS	F	df	MS	F
Sex	2	0.05	0.9 ns	2	0.16	1.8 ns	2	1.29	34.8*	2	1.04	27.6***	1	4.95	87.9**
Population	2	3.69	87.9***	2	2.80	62.5***	2	1.14	88.7***	2	0.19	3.1*	2	0.07	2.7†
Sex × population	4	0.06	1.4 ns	4	0.09	2.0†	4	0.04	2.8*	4	0.04	0.6 ns	2	0.06	2.1 ns
Error	171	0.04	...	171	0.04	...	171	0.01	...	171	0.06	...	114	0.03	...

Note. Analyses are model 3 ANOVAs with sex and population as fixed and random factors, respectively. Analyses correspond to data in table 3. ns = not significant.

† $P < 0.10$.
* $P < 0.05$.
** $P = 0.01$.
*** $P < 0.001$.

Sexual Dimorphism

Populations differed significantly for all traits that were examined (table 2). Overall, plants from WA tended to be the largest and those from YY the smallest (table 3). There were significant differences among the sexes for secondary sexual traits (flower diameter, ovary diameter, flower number), but not for leaf length or stem height (table 2). Males from YY and WH produced larger flowers than hermaphrodites, but in WA they did not differ. In all populations, flowers on males (polliniferous) and hermaphrodites (perfect) were 1.2–1.4 times larger than flowers on females (ovuliferous). Females produced flowers with ovaries that were 1.4–1.6 times larger than those on hermaphrodites in all populations. Males and hermaphrodites produced similar numbers of flowers and both produced more than females. All populations showed the same trend. The only significant sex population interaction was for flower diameter, which resulted from the overall smaller size of flowers at YY.

Total flower production per plant varied significantly among sexes, but not populations (sex: $F_{2,4} = 604.08$, $P < 0.001$; population: $F_{2,1161} = 1.47$, $P = 0.230$; fig. 2). The sex population interaction was not significant ($F_{4,1161} = 0.15$, $P = 0.965$). Males and hermaphrodites produced similar numbers of flowers (Tukey tests, all $P > 0.05$), and both produced ca. 1.4 times more flowers than females (Tukey tests, all $P < 0.05$). Females produced ca. 1.12 times more ovuliferous flowers than hermaphrodites in all populations (sex: $F_{1,2} = 1893.17$, $P < 0.001$; population: $F_{2,774} = 0.38$, $P = 0.684$; sex population: $F_{2,774} = 0.01$, $P = 0.988$; fig. 2).

Tepal and nectary sizes varied significantly among the sexes (tables 4, 5). Flowers on males had larger tepals than those on hermaphrodites and both were larger than those on females. Tepals on males and hermaphrodites were 2.1–2.2 and 1.5–1.9 times larger than those on females, respectively. For nectaries, those on males and hermaphrodites did not differ and were 1.5–1.9 times larger than those on females. Tepal size also differed among populations, and tended to be largest at WH and smallest at YY. Nectary size did not differ among populations. For tepals and nectaries, the sex population interaction was not significant, indicating that trends in sizes were consistent in all populations. Plants within populations, and the sex plants within-population interactions were significant.

Table 3

Comparison of Plant Traits of Males, Females, and Hermaphrodites

Population and sex	Leaf length	Inflorescence height	Flower diameter	Number of flowers	Ovary diameter
YY:					
Male	94.3 ± 4.2	69.1 ± 3.1	15.7 ± 0.3^A	3.0 ± 0.2^A	...
Female	105.5 ± 4.1	68.7 ± 3.8	11.3 ± 0.3^C	2.3 ± 0.1^B	2.9 ± 0.1^A
Hermaphrodite	95.2 ± 5.9	71.2 ± 2.9	13.7 ± 0.4^B	2.8 ± 0.2^A	2.0 ± 0.1^B
WH:					
Male	136.7 ± 5.5	101.9 ± 3.7	19.2 ± 0.3^A	3.3 ± 0.2^A	...
Female	130.3 ± 4.3	91.3 ± 4.7	14.7 ± 0.6^C	2.5 ± 0.5^B	2.9 ± 0.1^A
Hermaphrodite	133.5 ± 7.9	93.6 ± 4.2	17.5 ± 0.5^B	3.5 ± 0.2^A	1.8 ± 0.1^B
WA:					
Male	171.4 ± 8.6	115.1 ± 5.8	18.4 ± 0.4^A	3.2 ± 0.2^A	...
Female	156.3 ± 6.3	98.8 ± 4.2	14.6 ± 0.3^C	2.4 ± 0.2^B	2.6 ± 0.1^A
Hermaphrodite	151.8 ± 4.5	106.2 ± 4.6	18.8 ± 0.4^A	3.0 ± 0.2^A	1.8 ± 0.1^B

Note. Values are means ± SE, and measurements are in millimeters. In each of three populations, 20 plants of each sex were examined. Means with different superscripts within populations differed significantly (Tukey tests, $P < 0.05$). Analyses are given in table 2.
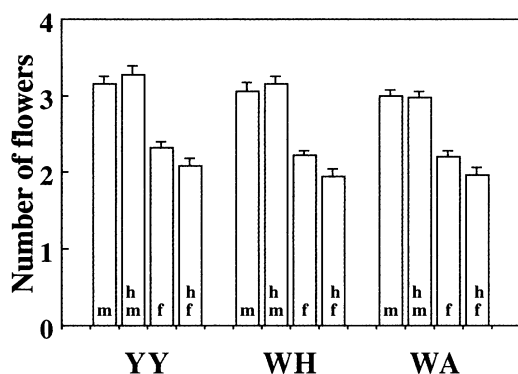
Fig. 2 Mean (±SE) number of flowers on male (m), female (f), and hermaphrodite plants (n = 130) in three populations of *Wurmbea dioica* (YY, WH, WA). For hermaphrodites, the total number of flowers (hm) and the number of ovuliferous flowers (hf) are presented. Males and hermaphrodites produced similar total numbers of flowers (P > 0.05), and both produced more than females (P < 0.05). Females produced more ovuliferous flowers than hermaphrodites (P < 0.001).

Gender Estimates

Functional femaleness (Gi) of hermaphrodites was similar in all populations ($F_{2,104}$ = 2.64, P = 0.077; fig. 3). All Gi estimates for hermaphrodites were less than 0.50, indicating that gender of these plants was male biased. Estimates of Gi for hermaphrodites were: YY, 0.32 ± 0.02 (range: 0.14–0.49); WH, 0.27 ± 0.02 (0.14–0.39); and WA, 0.32 ± 0.01 (0.21–0.44).

Self-Compatibility of Hermaphrodites

In each population, all self-pollinated flowers produced seeds. Percentage self seed set did not differ among populations and was ca. 21% overall (YY: 25.0% ± 4.3%; WH: 16.1% ± 4.0%; WA: 22.9% ± 4.8%; $F_{2,18}$ = 1.42, P = 0.27).

Discussion

Our findings corroborate and extend those of Barrett et al. (1999) concerning sex expression and gender modification in subdioecious populations of *Wurmbea dioica*. We found that 71% of males were labile in their sex expression, producing perfect flowers under favorable conditions, whereas female sex expression was constant. We also found greater ovule and seed production by females compared with hermaphrodites and patterns of sexual dimorphism among the three phenotypes

that were consistent with theoretical models that predict the maintenance of females in cosexual populations (Charlesworth 1999). Finally, males produced more pollen than hermaphrodites, indicating possible resource-based trade-offs between male and female functions when males produced functional pistils. Below, we examine sex expression and sexual dimorphism to provide insight into the stability of subdioecy in *W. dioica*.

The three *W. dioica* populations studied here were subdioecious and were near the dioecious end of hermaphrodite-gynodioecy-dioecy continuum (Lloyd 1976; Ross 1982; Webb 1999). Sex ratios were slightly male biased, and frequencies of male, female, and hermaphrodite phenotypes pooled over populations were ≈50%, 43%, and 7%, respectively. Our study populations near Melbourne experience predictable and abundant winter rainfall, and the presence and frequency of hermaphrodites were consistent with Barrett's (1992) previous study of sex ratio variation in southern Australia. However, we examined sex ratios for 1 yr only, and the frequency of hermaphrodites may vary in different years depending on conditions prior to flowering. For example, frequencies of hermaphrodites can exceed 20% following good conditions in some populations (Barrett et al. 1999; M. Ramsey, unpublished data). This indicates the plasticity of this sexual system in *W. dioica* and the importance of long term studies in assessing the sex ratios in subdioecious populations.

Barrett et al. (1999) were the first to report gender modification in *W. dioica*. In a field demographic study over two consecutive flowering seasons, males but not females exhibited labile sex expression, and 30% of marked males became hermaphrodites following a winter of abundant rainfall. Our findings from the glasshouse corroborate the presence of sex lability in *W. dioica*. We found that female sex expression was invariable (canalized) but that 71% of males produced at least one perfect flower over a 3-yr period under favorable glasshouse conditions. Hermaphrodites maintained their phenotype and did not revert to males. This indicates that male phenotypes respond to favorable environmental conditions by producing functional pistils and that, if maintained under good conditions, they will continue to produce perfect flowers. Although 71% of males were labile, 29% of males produced polliniferous flowers only. Whether sex expression in these latter males is canalized or whether there is variation among males for pistil production based on a resource-threshold system that is under genetic control remains to be determined (Delph and Lloyd 1991; McArthur et al. 1992).

Estimates of functional femaleness of hermaphrodites in the field were similar in the three populations and indicate a trend

Table 4

Comparison of Petal and Nectary Areas of Males, Females, and Hermaphrodites

	Petals			Nectaries		
Population	Male	Female	Hermaphrodite	Male	Female	Hermaphrodite
YY	20.8 ± 0.9[A]	10.0 ± 0.4[C]	15.2 ± 0.6[B]	1.26 ± 0.04[A]	0.65 ± 0.03[B]	1.12 ± 0.05[A]
WH	24.7 ± 0.5[A]	11.8 ± 0.6[C]	21.7 ± 1.2[B]	1.02 ± 0.04[A]	0.65 ± 0.03[B]	1.11 ± 0.07[A]
WA	22.1 ± 0.6[A]	10.2 ± 0.5[C]	19.5 ± 0.5[B]	0.95 ± 0.05[A]	0.64 ± 0.04[C]	1.02 ± 0.04[A]

Note. Values are means ± SE in square millimeters. Means with different superscripts within populations differed significantly (Tukey test, P < 0.05).

Table 5

Effects of Sex, Population, and Plant on Petal and Nectary Areas

Source	df	Petals		Nectaries	
		MS	F	MS	F
Sex	2	15.72	90.9***	8.83	44.8***
Population	2	1.28	9.2***	0.59	2.35 ns
Plants (population)	30	0.14	13.1***	0.25	12.3***
Sex × population	4	0.17	1.2 ns	0.20	1.00 ns
Sex × plant (population)	60	0.15	14.0***	0.20	9.9***
Error	198	0.01	...	0.02	...

Note. Analyses are model 3 partially hierarchical ANOVAs with sex as a fixed, crossed factor and population and plants nested within population as random factors. For each sex, three petals on each of 11 plants in each of three populations were measured. ns = not significant.

*** $P < 0.001$.

toward maleness (overall mean, $G = 0.31 \pm 0.01$, $n = 105$). They probably underestimate maleness because hermaphrodites may not produce flowers with functional pistils in all flowering seasons, thus reducing G over an individual's lifetime. If selfing and inbreeding depression occurs, then G could be reduced further. Although hermaphrodites achieve most of their fitness through male function, femaleness can be modified by environmental conditions. Following continual applications of water and fertilizer in the glasshouse, the number of ovuliferous flowers produced by hermaphrodites can be increased, and plants can flower consistently as hermaphrodites (M. Ramsey, unpublished data). As suggested by Barrett (1992), the presence of constant females and inconstant and possibly constant males indicates that sex expression in *W. dioica* is complex and influenced by genetic and environmental factors. Similar complexity has been reported for other diclinous species (Lloyd and Bawa 1984; Delph and Lloyd 1991; Wolfe and Shmida 1997).

In the three *W. dioica* populations studied here, females produced 1.3 times more ovules per flower and 2.5–3.7 times more open-pollinated seeds per plant than hermaphrodites. Providing that these large fertility differences reflect lifetime differential fitness between females and hermaphrodites, they are sufficient to maintain females in these subdioecious populations (Charlesworth and Charlesworth 1978; Charlesworth 1999). Some of the seed fertility advantage was due to females producing more ovules per flower and more ovuliferous flowers per plant than hermaphrodites (mean of three populations: ovules, 63.5 vs. 48.2; flowers, 2.3 vs. 2.0). However, the major factor that contributed to the fecundity advantage of females was the low conversion of ovules to seeds caused by greater seed abortion in hermaphrodites compared with females (percentage seed set: 47% vs. 23%; percentage seed abortion: 21% vs. 51%). Differences in ovule and seed production may reflect genetic differences in resource allocation patterns between females and hermaphrodites that may have evolved to maximize fitness. Such differences may result from the spread of genetic modifiers that reduce female function in hermaphrodites and facilitate increases in male function (Charlesworth and Charlesworth 1978). The threefold difference in seed produc-

tion between females and hermaphrodites in this study was greater than that reported by Barrett et al. (1999), who found that seed production of hermaphrodites was 70% that of females. This may reflect site differences that influence the production of perfect flowers or seeds per flower by hermaphrodites. Similar differences in ovule and seed production to those found here have been reported in other diclinous species (e.g., Sakai and Weller 1991).

Other factors that could contribute to the low open-pollinated seed set of hermaphrodites relative to females include pollen limitation, resource limitation, or early-acting inbreeding depression following selfing. Of these factors, self-fertilization and inbreeding depression are considered major factors influencing the evolution of dicliny (Charlesworth 1999), and high levels of inbreeding depression have been reported in several gynodioecious and subdioecious species (Sakai et al. 1989, 1997; Delph and Lloyd 1996; Schultz and Ganders 1996). We found that hermaphrodites in the study populations were partially self-compatible and selfed seed set was similar to open-pollinated seed set (percentage seed set: 21% vs. 23%). Further studies examining selfing rates and inbreeding depression of hermaphrodites are required to clarify the relative importance of inbreeding depression in the evolution of subdioecy in *W. dioica* (e.g., Sakai et al. 1997).

Trade-offs between male and female allocation of resources to reproduction underlie resource compensation models in the evolution of diclinous mating systems from hermaphrodite ancestors (Charnov 1982; Geber 1999). In previous work on *W. dioica*, Barrett (1992) examined pollen production of hermaphrodites from a monomorphic population in southwestern Australia and males from dimorphic populations in southeastern Australia. Males produced less pollen than hermaphrodites. In this study, males produced 1.3 times more pollen that was 7% more viable than that from hermaphrodites. Because hermaphrodites are inconstant males, our results indicate
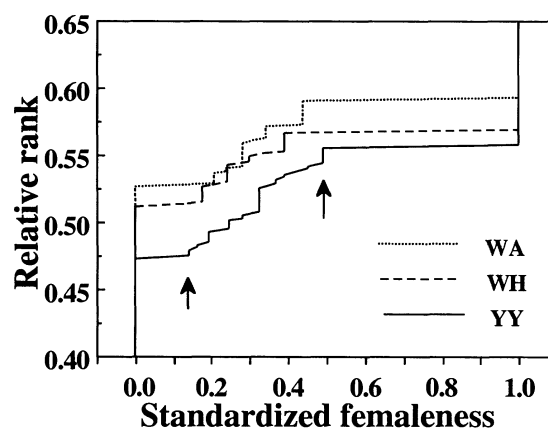

Fig. 3 Distributions of functional gender of male, female, and hermaphrodite plants in three populations of *Wurmbea dioica* (YY, WH, WA). The percentage of hermaphrodites in populations ranged from 6% to 8%. Distributions are the relative ranks of standardized femaleness of plants. Values range from 0 (males) to 1 (females). The Y-axis has been truncated for comparison of distributions. Arrows denote the range of estimates for hermaphrodites. Between 495 and 561 plants were examined in each population.

that pollen production is reduced when functional pistils are produced, supporting the concept of resource-based trade-offs. Whether our findings reflect negative genetic correlations between male and female functions, however, is unknown (Atlan et al. 1992; Ashman 1999; Olson and Antonovics 2000). Only a few studies have found differences in pollen or stamen production between hermaphrodites and males (Wolfe and Shmida 1997). Such trade-offs imply that for sex lability to be adaptive, fitness gains through seeds must outweigh the fitness losses of producing less pollen. If trade-offs can be modified with increasing resource availability such that differences in pollen production are negated, then producing seeds would augment overall plant fitness, providing that seed production does not affect survival or future reproduction. Producing seeds may be particularly advantageous if resource availability varies among years and greater resources result in increased pollen production by males, causing male fitness gain curves to decelerate (Seger and Eckhart 1996).

Males and hermaphrodites produced more flowers and larger flowers than females in all three *W. dioica* populations. Sexual dimorphism for flower number and size has been found in many of the dimorphic *W. dioica* populations that have been studied to date (Barrett 1992; Vaughton and Ramsey 1998; Barrett et al. 1999). In temperate animal-pollinated diclinous species, the polliniferous phenotypes often produce more and larger flowers than females (Delph et al. 1996). A functional theory for this dimorphism is based on the premise that males allocate more resources to attract pollinators for effective pollen dispersal because their mating success is limited by access to mates, whereas female success is limited by resources (Bateman 1948; Bell 1985; Delph et al. 1996). This sexual selection explanation is consistent with the available data for *W. dioica*. At the YY site, pollinators visited males more frequently than females, and plants with more or larger flowers were visited more frequently than smaller plants. Also, pollen of males with larger flowers was removed more rapidly than those with smaller flowers, whereas seed set of females was negatively related to flower size, indicating potential trade-offs between pollinator attraction and female fecundity (Vaughton and

Ramsey 1998). In this study, males produced larger flowers than hermaphrodites in two of the three populations, although the size differences were less than those between hermaphrodites and females. The male versus hermaphrodite size differences may reflect trade-offs between attraction and female function within hermaphrodites. Collectively, these results indicate that greater flower size is probably advantageous for males but not for females (Bell 1985; Eckhart 1991; Delph et al. 1996).

Males and hermaphrodites produced similar numbers of flowers, which contrasts with Barrett et al. (1999), who found that hermaphrodites produced more flowers than did males. In both studies, hermaphrodites produced more flowers in total than females, although females produced more ovuliferous flowers. The number of ovuliferous flowers on hermaphrodites, however, can be increased under favorable watering and fertilizer regimes in the glasshouse (M. Ramsey, unpublished data). These findings indicate that hermaphrodite flowering and fruiting patterns may be closely linked to plant size, plant vigor, or resource status as suggested by Barrett et al. (1999). Size- or resource-dependent gender modification is consistent with the proposal that genetic modifiers regulate pistil production by hermaphrodites depending on resource thresholds (Delph and Lloyd 1991). Whether the production of perfect flowers by inconstant males (i.e., sex lability) is adaptive or developmental noise, and whether subdioecy is stable, remain to be addressed in *W. dioica*.

Acknowledgments

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