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Evidence of gynodioecy and sex ratio variation in *Wurmbea biglandulosa* (Colchicaceae)

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Abstract. A valuable approach to understanding the evolution of gender dimorphism involves studies of single species that exhibit intraspecific variation in sexual systems. Here we survey sex ratios in 35 populations of *Wurmbea biglandulosa*, previously described as hermaphroditic. We found pronounced intraspecific variation in sexual systems; populations in the northeastern part of the species' range were hermaphroditic, whereas other populations were gynodioecious and contained 2–44% females. Populations with lower annual rainfall were more likely to be gynodioecious, supporting the view that gender dimorphism evolves more frequently in harsher environments. In gynodioecious populations, however, female frequency was not related to either annual rainfall or habitat, indicating that other factors are important in determining sex ratio variation. Females had smaller flowers and shorter stems than did hermaphrodites, potentially providing a basis for resource compensation. A female fecundity advantage may contribute to the maintenance of females in populations because females produced more ovuliferous flowers and had more ovules per flower than did hermaphrodites.

Key words: Functional gender, gender expression, geographic variation, gynodioecy, plant breeding systems, sex ratios, sexual dimorphism, *Wurmbea biglandulosa*.

Introduction

Gynodioecy is a dimorphic sexual system in which populations contain females (male steriles) and hermaphrodites. Hermaphrodites contribute genes to the next generation through pollen and ovules, whereas females reproduce only via ovules. The establishment of females in populations requires that they have greater seed fitness than hermaphrodites to compensate for the absence of male function. The required female fitness advantage depends upon the mode of inheritance of male sterility. Under nuclear control females must have a two-fold advantage in seed fitness to establish, whereas if male sterility is under nuclear-cytoplasmic control, then a less than two-fold advantage is required (Lewis 1941, Charlesworth and Charlesworth 1978). Greater relative seed fitness of females may have two sources. First, by not producing pollen females may have more resources to devote to lifetime seed production (i.e. resource compensation, e.g. Eckhart 1992; Ashman 1994, 1999; Wolfe and Shmida 1997). Second, females may produce higher quality seeds than hermaphrodites if the latter are self-compatible and exhibit inbreeding depression (e.g. Kohn and Biardi 1995, Schultz and Ganders 1996, Sakai et al. 1997).

A feature of gynodioecious species is the often extremely wide variation in female frequencies among populations (reviews by Lloyd 1976, Couvet et al. 1990, Webb 1999). Explanations for this variation have focused on the relative seed fitness of the two sexual morphs and the genetic control of male sterility (Lewis 1941, Lloyd 1976, Charlesworth 1981, Couvet et al. 1990, Webb 1999). Assuming nuclear control, theory predicts that as the frequency of females increases to a maximum of 0.5 the relative seed fitness of the hermaphrodites will decrease to zero (Lloyd 1976). This is consistent with observed sex ratio variation in several gynodioecious species (Webb 1979, Delph 1990a, Ashman 1999). When male sterility is under nuclear-cytoplasmic control the situation is more complex and females may be maintained at a higher frequency than would be expected based on their seed fitness advantage alone (Charlesworth 1981, Frank 1989, Gouyon et al. 1991). In such species, female frequencies (p_f) are often very variable and may exceed 50%. Examples include: *Thymus vulgaris* ($p_f = 5$–95%, Dommée et al. 1978), *Plantago lanceolata* ($p_f = 0$–70%, Ross 1969), *Origanum vulgare* ($p_f = 1$–62%, Kheyr-Pour 1980); and *Silene acaulis* ($p_f = 50$–80%, Hermanutz and Innes 1994; $p_f = 23$–39%, Delph and Carroll 2001).

There is growing recognition that ecological factors, particularly environmental stress, may ease the evolution of gender dimorphism by altering the relative seed fitness of the sexual morphs. Several demographic studies have shown that harsher environments are more likely to contain dimorphic populations (Delph 1990b, Barrett 1992, Costich 1995, Case and Barrett 2001) and higher frequencies of females (Delph 1990a, Ashman 1999, Alonso and Herrera 2001, Delph and Carroll 2001). Similary, an association between dry habitats and the evolution of gender dimorphism has been reported in several phylogenetic studies (Hart 1985, Weller et al. 1995). Increased gender dimorphism in harsh environments may arise because reproduction of hermaphrodites, which must bear the costs of producing both seeds and pollen, is more resource-limited than that of females. Because resource limitation is likely to be most pronounced in harsh environments, relative seed fitness of females should be greatest in this situation (e.g. Delph and Carroll 2001). A greater female seed fitness advantage may also arise in harsh environments if pollination differs spatially, resulting in an increase in selfing and inbreeding in hermaphrodites (e.g. Delph 1990b, Weller et al. 1995).

A valuable approach to understanding the role of ecological factors in the evolution of gender dimorphism involves studies of single species that exhibit intraspecific variation in sexual systems (reviewed by Barrett et al. 2001). Such studies are not complicated by phylogenetic considerations and populations of the same species should be similar in their morphology and ecology. Further, examination of a single species over a wide geographical area can provide insight into the importance of ecological gradients on the invasion of unisexual phenotypes (e.g. Costich 1995, Sarkissian et al. 2001). Intraspecific variation in sexual systems, however, is poorly documented and in order to find new study species, broad-scale surveys of sex ratios over a wide geographic area or along environmental gradients are required.

Wurmbea (Colchicaceae) is a genus of small, perennial geophytes native to southern Africa and southern Australia. Australian species comprise hermaphroditic (monomorphic) and dimorphic taxa (Macfarlane 1980). Extensive intraspecific variation in sexual systems has been documented in *W. dioica* ranging from hermaphroditism to dioecy through varying degrees of gynodioecy and subdioecy (Barrett 1992). Here we undertake a broad-scale survey of sex ratios in 35 populations of *W. biglandulosa*, a species that has been previously reported as hermaphroditic (Macfarlane 1980). We document intraspecific variation in sexual systems and examine the influence of rainfall and habitat on the occurrence of gender dimorphism and variation in female frequencies among populations. We also examine gender differences in flower number, ovule production, flower diameter and stem height to gain insight into the factors that maintain females in populations at a broad geographic scale.

Materials and methods

Study species and sites. *Wurmbea biglandulosa* (R. Br.) T.D. Macfarl. ssp. *biglandulosa* occurs in a variety of habitats in eastern Australia from southeastern Queensland to northeastern Victoria. Flowering plants have a corm, a single unbranched stem, 2–3 annual leaves, and an inflorescence spike with 1–6 flowers. Flowering occurs in spring (August–October) and flowers are insect-pollinated. Flowers have six white ovate tepals (8–10 mm long), each with two nectaries located towards the base (Macfarlane 1980).

Sex ratios. We assessed sex ratios in 35 *W. biglandulosa* populations selected to include most of the species' range and a variety of local habitats occupied by the species (Table 1, Fig. 1). Populations were at least 1 km apart and sex ratios were scored during peak flowering in 1998–2000. The sex of at least 500 plants, or all plants if the population size was less than 500, was recorded while walking transects through populations. Only plants with all their flowers open were scored. In *W. biglandulosa*, polliniferous plants are sexually labile and flower as males or hermaphrodites depending on cirumstances (G. Vaughton and M. Ramsey unpublished). We classified plants as males if they had polliniferous only flowers and as hermaphrodites if they had at least one perfect flower. Plants were classified as females if their flowers had a functional ovary but nonfunctional stamens. Most flowers on females have neither filaments nor anthers, although occasional flowers have shortened filaments, and rare flowers have shortened filaments and shrivelled anthers with no pollen ($<5\%$ of plants). A χ^2 test was used to examine heterogeneity in female frequency among gynodioecious populations.

We carefully examined plant phenotypes in the study populations to ensure that they were *W. biglandulosa*. In general, we avoided surveying populations where *W. biglandulosa* might be hybridizing with subdioecious *W. dioica*, the range of which overlaps with *W. biglandulosa*. However, we specifically surveyed one population (Burrowa Pine, population 35) in which hybridization between the two *Wurmbea* species probably occurs because we were interested in the effect on sex ratios of *W. biglandulosa*. In this population we scored the sex of recognizable *W. biglandulosa* phenotypes and *W. dioica* phenotypes separately.

Ecological correlates. We assessed whether gynodioecy was associated with average annual rainfall using a logistic regression assuming a binomial error distribution (Collett 1991). Rainfall (mm) was the continuous predictor variable and the presence or absence of females was the discrete response variable. Rainfall data were obtained from the nearest weather station to each population (Bureau of Meteorology, Australia). Annual rainfall decreases from east to west across the study area and the incidence of winter rainfall increases from north to south (Fig. 1).

For each population we categorized the local habitat as granite outcrop, grassland, forest or creekbank (Table 1). We assessed whether sexual system (gynodioecy vs. hermaphroditism was associated with habitat (granite outcrop vs. grassland) using a 2×2 contingency analysis. We assumed that granite outcrops represented a less favourable habitat because plants are smaller on granite compared with grassland (G. Vaughton and M. Ramsey unpublished data). Forest and creekbank habitats occurred infrequently and were not included in the analysis (Table 1).

To assess the role of habitat and average annual rainfall on female frequency in gynodioecious populations we used an ANCOVA with habitat as a fixed factor and average annual rainfall as the covariate. Forest and creekbank habitats were not included in the analysis. The habitat × rainfall interaction was not significant ($P > 0.50$) and was omitted from the final model.

Gender estimates. We quantified the functional gender of plants in hermaphroditic and gynodioecious populations by calculating standardized phenotypic gender (*Gp*, Lloyd 1980). The method records an individual's expenditure on pollen and ovules (or seeds) relative to the population expenditure. Here $Gp = di/(di + liE)$, where *di* and *li* are the number of ovuliferous flowers and polliniferous flowers, respectively, produced by plant *i*. The equivalence factor, $E = \sum di / \sum li$, measures the ratio of ovuliferous to polliniferous flowers in the population as a whole. A *Gp* value of 1.0 represents plants that only produce ovules and $Gp = 0.0$ represents plants that only produce pollen.

Table 1. The frequency of the three sex phenotypes in 35 populations of *Wurmbea biglandulosa* in southeastern Australia. Habitat and the number of plants sampled in each population are also given. Locations of populations are shown in Fig. 1

	Population	Habitat[‡]	Number of plants	Frequency of females	Frequency of hermaphrodites	Frequency of males
1	Stanthorpe	1	1028	0.00	0.98	0.02
2	Tooloom	1	202	0.00	0.96	0.04
3	Tenterfield	2	764	0.00	0.97	0.03
4	Sundown East	2	608	0.00	0.93	0.07
5	Emmaville	2	1140	0.00	0.98	0.02
6	Tinga	2	643	0.00	0.97	0.03
7	Mt Yarrowyck	1	233	0.00	0.89	0.11
8	Rocky River	2	1243	0.01	0.95	0.04
9	Goonoowigall	1	1151	0.18	0.71	0.11
10	Linton	2	241	0.15	0.81	0.04
11	Warabah	1	537	0.20	0.71	0.09
12	Moonbi	1	514	0.44	0.52	0.04
13	Woodsreef	2	583	0.34	0.64	0.02
14	Horton Falls	1	562	0.04	0.83	0.13
15	Glacial area	1	1141	0.05	0.87	0.08
16	Mt Kaputar	1	508	0.04	0.89	0.07
17	Watertower	2	507	0.12	0.84	0.04
18	Camp Elongery	2	1053	0.16	0.81	0.03
19	Timor Rock	2	564	0.10	0.84	0.06
20	Purlewaugh	2	558	0.04	0.94	0.02
21	Pandora's Pass	2	711	0.06	0.89	0.05
22	Joshua's Leap	2	521	0.05	0.92	0.03
23	Gungal	1	248	0.05	0.91	0.04
24	Ulan	4	588	0.20	0.74	0.06
25	Goolma	2	532	0.08	0.86	0.06
26	Wellington	2	555	0.09	0.86	0.05
27	Nangar 1	2	605	0.07	0.87	0.06
28	Nangar 2	2	528	0.11	0.85	0.04
29	Geehi	3	733	0.16	0.76	0.08
30	Grassy Flat	3	1061	0.16	0.73	0.11
31	Tom Groggin	3	1232	0.20	0.69	0.11
32	Mt Pilot Summit	1	529	0.02	0.88	0.10
33	Mt Pilot Dam	1	99	0.08	0.89	0.03
34	Beechworth	1	538	0.04	0.93	0.03
35	Burrowa Pine	2	176	0.35	0.21	0.44

[‡] [1] Granite Outcrop; [2] Disturbed grassland; [3] Forest; [4] Creekbank

Hermaphrodites produce both pollen and ovules and G_p varies depending upon the frequency of females in the population (Lloyd 1980).

For gender estimates, we scored the number of ovuliferous and polliniferous flowers on 100 plants in the Tenterfield, Beechworth, Ulan and Moonbi populations (3, 34, 24, 12; see Table 1) that illustrate the range of female frequencies. In each population, sexual phenotypes were scored in proportion to their frequency in populations as determined by surveys of sex ratios described above. The ranks of the *Gp* values were plotted as frequency distributions.

We were also interested in whether populations with higher female frequencies had hermaphrodites with lower allocation to female function as indicated by ovuliferous flower production. We used a

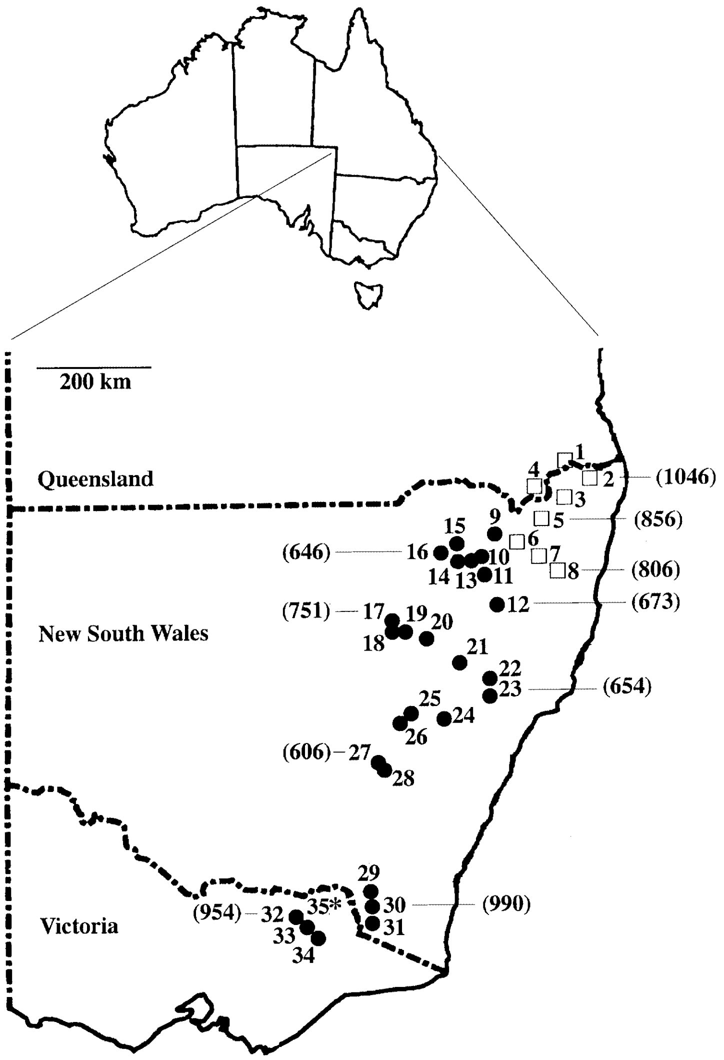

Fig. 1. The locations of 35 *Wurmbea biglandulosa* populations surveyed for sex ratios in southeastern Australia. Hermaphrodite populations (open squares) were located in the northeastern part of the species' range. Gynodioecious populations (filled circles) were located in the western, central and southern parts of the species' range. In one population (asterisk) *W. biglandulosa* was probably hybridizing with *W. dioica*. Average annual rainfall for selected populations are given in parentheses. Population numbers and sex ratios are given in Table 1

Pearson correlation to examine the association between female frequency and the ratio of the number of ovuliferous flowers produced by hermaphrodites to that of females.

Sexual dimorphism. We examined sexual dimorphism between females and hermaphrodites in gynodioecious populations by assessing flower production, ovule production, flower diameter and stem height. Flower diameter and stem height contribute to the overall showiness of the floral display and we assessed sexual dimorphism in these traits to determine whether hermaphrodites allocated more resources to pollinator attraction than females. Because polliniferous phenotypes are sex labile we included males in our sample of hermaphrodites in proportion to their frequency in the total pool of polliniferous plants (Table 1).

For flower production we counted the total number of flowers and the number of ovuliferous flowers on 50 plants of each sex in all gynodioecious populations. Where there were fewer than 50 females in populations we counted flowers on all available female plants.

We examined ovule production, flower diameter and stem height of females and hermaphrodites using 20 plants of each sex in each of 14 gynodioecious populations (9, 10, 12, 13, 14, 15, 16, 18, 19, 22, 24, 28, 30, 34; see Table 1). These populations were chosen to cover the geographic range of gynodioecy and included the observed range in female frequencies (Fig. 1). For ovule production we dissected ovaries of the first flower on inflorescences and counted ovules using a stereo microscope. The diameter of flowers with fully expanded tepals was measured to the nearest 0.1 mm with vernier callipers as the distance between the tips of two opposite tepals (mean of two measurements) on the first flower. Stem height was measured to the nearest 1 mm with a ruler as the distance from the soil to the base of the first flower.

Sexual dimorphism in plant traits was examined using factorial ANOVAs with sex and geographic region as fixed factors and population as a random factor nested within geographical region. Geographic region was included to control for variation in the amount and timing of rainfall and significant topographical features. Regions included the Nandewar Range (North Western Slopes Botanical Division, hereafter R1; populations 9–16), the Warrumbungle Range (North Western Slopes, R2; 17–20), the Central Western Slopes (R3; 21–28) and the NSW Southern Tablelands and Victorian Eastern Highlands (R4; 29–34). For flowers there were 4–8 populations in each region and for the other traits 2–7 populations in each region. It was not possible to include habitat as a factor in the analysis because granite outcrops were not represented in regions 2 and 3.

Statistical analyses. Numbers of flowers (+1) and ovules, flower diameter and stem height were transformed using natural logarithms. Transformation improved normality and homoscedasticity as established by Shapiro-Wilk and Levene's tests, respectively. For ANOVAs, Type III sums of squares were calculated. Analyses were performed using Minitab (10Xtra, Minitab Inc., State College, PA) or JMP (3.1, SAS Institute Inc., Cary, NC). Population 35 was omitted from all statistical analyses because of hybridization with *W. dioica*.

Results

Sex ratio. Populations in the northeastern part of the species' range (populations 1–8) were hermaphroditic and contained no females or less than 1% females (Fig. 1). In contrast, populations in the northwestern, central and southern parts of the species' range (populations 9–34) were gynodioecious (Fig. 1). In these populations, female frequencies varied from 2% to 44% and were significantly heterogeneous ($\chi^2 = 1184.90$, df = 25, $P < 0.001$, Table 1).

The frequency of hermaphrodite phenotypes in hermaphroditic and gynodioecious populations ranged from 52% to 98% (Table 1). Hermaphrodites comprised several forms; plants in which the lower flowers were perfect, the most common phenotype, and plants in which the lower flowers were perfect but the terminal flower(s) were male. In all populations there was a low frequency of male phenotypes (2–13%) that produced polliniferous only flowers (Table 1). Males were uniformly small in size and usually produced only one or occasionally two flowers (mean ± SE, 1.27 ± 0.01, n = 1270).

In the Burrowa Pine population where *W. biglandulosa* hybridized with *W. dioica*, the percentage of females was 35% which was in the upper range of female frequencies observed in other *W. biglandulosa* populations. Male frequencies were 44% and were higher than all other populations surveyed. Unlike the other populations, males were similar in size to hermaphrodites and had two or three flowers (mean ± SE, 2.20 ± 0.06, n = 78). Collectively, these observations indicate introgression of genes from *W. dioica* to *W. biglandulosa*. Sex ratios of *W. dioica* phenotypes at Burrowa Pine were similar to previous reports for this species in high rainfall areas of eastern Australia (hermaphrodites, 23.0%; males, 45.3%; females, 31.7%; n = 126; Barrett 1992, Ramsey and Vaughton 2001).

Ecological correlates. Sexual system was significantly related to annual rainfall (logistic regression, $\chi^2 = 3.96$, df = 1, $P = 0.047$). Gynodioecy was more prevalent in areas of low annual precipitation. No hermaphrodite populations were found in areas with annual rainfall of less than 750 mm, whereas 50% of gynodioecious populations occurred in such areas.

Sexual system was not dependent on whether populations occurred on granite outcrops or in grasslands (Table 1, $\chi^2 = 0.68$, df = 1, $P = 0.409$). In gynodioecious populations, female frequency was not related to either habitat ($F_{1,18} = 0.02$, $P = 0.891$) or the covariate, average annual rainfall ($F_{1,18} = 1.16$, $P = 0.294$).

Gender estimates. The four populations chosen to illustrate the range in patterns of functional femaleness in *W. biglandulosa* were Tenterfield ($p_f = 0.0\%$), Beechworth ($p_f = 4.5\%$), Ulan ($p_f = 20.2\%$) and Moonbi ($p_f = 43.8\%$, Fig. 2). The *Gp* estimate for hermaphrodites in the gynodioecious populations were 0.492 ± 0.004 at Beechworth (range 0.34–0.50, n = 93), 0.425 ± 0.008 at Ulan (range 0.31–0.47, n = 74) and 0.326 ± 0.009 at Moonbi (range 0.23–0.37, n = 52). Variation in functional gender of hermaphrodites was similar in populations of both sexual systems. The somewhat lower variation in the Beechworth population was associated with moist soils and increased femaleness of the hermaphrodites.

Female frequency in populations was negatively related with female allocation in hermaphrodites as indicated by the production of ovuliferous flowers but the relationship was not significant ($r = -0.156$, $t_{24} = 0.77$, $P = 0.447$).

Sexual dimorphism. Females produced significantly more total flowers, ovuliferous flowers and ovules per flower than did hermaphrodites (Table 2, Fig. 3A, B). For ovuliferous flowers, the gender × region interaction was significant; females produced more ovuliferous flower in all regions (Tukey test, $P < 0.05$), but the extent of the female advantage varied among regions (female:hermaphrodite ratio, 1.19–1.35). Pooled over regions, females produced on average 1.06 times as many total flowers, 1.28 times as many ovuliferous flowers and 1.22 times as many ovules per flower as did hermaphrodites.

Females produced both significantly smaller flowers and shorter stems than did hermaphrodites (Table 2, Fig 3C, D). For flower diameter, the gender × region interaction was significant; females had smaller flowers in all regions (Tukey test, $P < 0.5$) but the f:h ratio varied among regions (0.74–0.84). Pooled over regions average flower diameter and stem height of females were 0.80 and 0.87 that of hermaphrodites, respectively.

There were significant or near significant effects of geographic region on ovuliferous flower and ovule production, flower diameter and stem height (Table 2). The magnitude of these traits increased from northern regions to southern regions (Fig. 3). In all analyses, there was also significant variation among populations within regions, which was related in part to differences in local habitat (Table 1).

Discussion

Our survey of *Wurmbea biglandulosa* populations over a wide geographic area revealed intraspecific variation in sexual systems and variation in population sex ratios. Populations of *W. biglandulosa* were either hermaphroditic or gynodioecious and in the latter female frequencies varied from 2–44%. Gynodioecy has not been previously reported in *W. biglandulosa*; previous taxonomic studies have classified *W. biglandulosa* as hermaphroditic (Macfarlane 1980). Our findings are significant because relatively few taxa with intraspecific variation in sexual systems have been identified. Such taxa provide valuable models for examining hypotheses proposed to explain the evolution of gender dimorphism (reviewed by Barrett et al. 2001).

Gynodioecy in *W. biglandulosa* is unlikely to be the result of recent introgression of male sterility genes from co-occurring subdioecious *W. dioica*. We recorded male sterility in numerous *W. biglandulosa* populations over a wide geographic area and it is unlikely that chance introgression could account for our findings on such a large scale. Further, we examined the Burrowa Pine population where probable hybridization between the two species was occurring. Sex ratios in this population were distinct from the other populations that we examined and contained high frequencies of both males and females. In addition, unlike the other populations males and hermaphrodites were similar in size. Collectively, these findings indicate probable gene flow from *W. dioica* at Burrowa Pine but not the other populations. The importance of introgression in the distant past or polyploidy (Miller and Venable 2000) in the evolution of gynodioecy in *W. biglandulosa* remains to be determined.

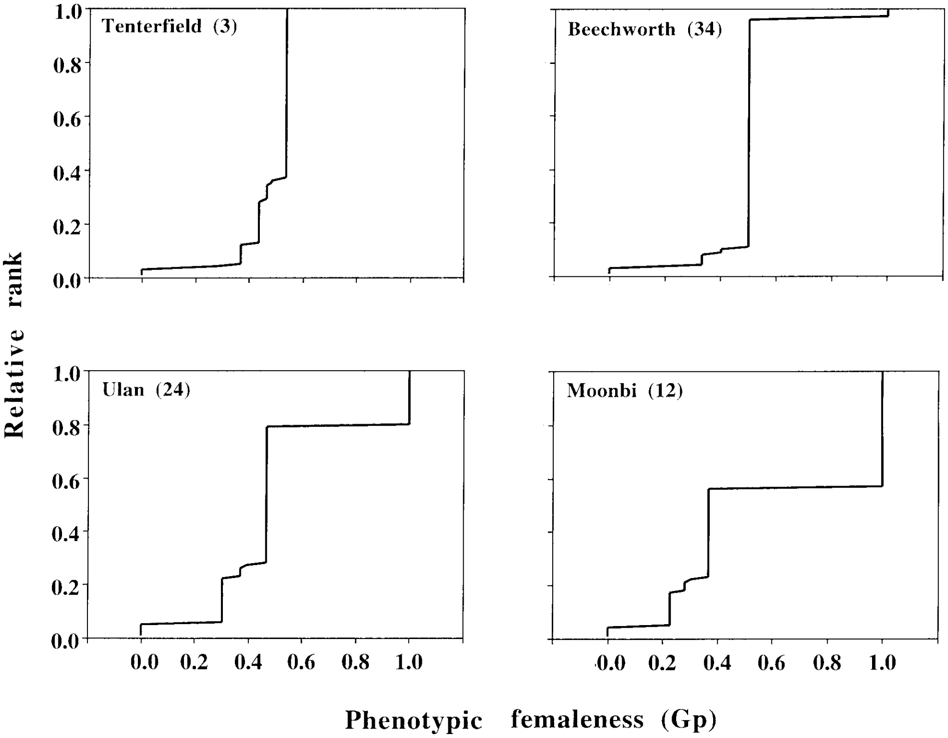

Fig. 2. Distributions of functional gender (G_P) in four populations of *Wurmbea biglandulosa.* Female frequencies were 0.0%, 4.5%, 20.2%, and 43.8% in the Tenterfield (3), Beechworth (34), Ulan (24), and Moonbi (12) populations, respectively. Distributions are the relative ranks of standardized femaleness of plants. Values range from 0 (males) to 1 (females). In each population 100 plants were examined

Theory predicts that females should more easily establish in harsh environments because it is in such situations that they are likely to achieve the necessary seed fitness advantage to compensate for their lack of male function (Charlesworth and Charlesworth 1978). We found some support for this view because *W. biglandulosa* populations with different sexual systems were geographically segregated and gynodioecy was more prevalent in areas of low annual rainfall. About 50% of gynodioecious populations occurred where the average annual rainfall was less than 750 mm, whereas no hermaphrodite populations were found in such areas. Studies of *W. dioica* in southwest Australia have also revealed intraspecific variation in sexual systems and an association between gender dimorphism and aridity. In *W. dioica* hermaphroditic and dimorphic populations are not geographically segregated but there is marked segregation in sympatry with hermaphroditic populations occupying moist microsites and dimorphic populations occupying dry microsites (Barrett 1992, Case and Barrett 2001). Similar findings have been reported in other genera. Monoecious subspecies of *Ecballium elaterium* on the Iberian Peninsula are restricted to the wetter northern region while dioecious subspecies occur only in drier areas of the south (Costich 1995). Similarly, dimorphic species of Hawaiian *Schiedea* are found in dry habitats, whereas most monomorphic species occur in wet habitats (Weller et al. 1995).

Although there was a greater incidence of gynodioecy in drier sites we found no evidence that female frequency was higher in such sites. This suggests that any female fitness advantage in drier sites was insufficient to allow an increase in the prevalence of females. In contrast, females are more common in poorer sites in *Hebe strictissima* (Delph 1990a),

Table 2. ANOVAs for the effects of sex (fixed factor; hermaphrodite vs. female plants), geographic region (fixed) and population nested within region (random) on the numbers of total and ovuliferous flowers, the number of ovules per ovuliferous flower, flower diameter and stem height in gynodioecious populations of *Wurmbea biglandulosa*

Source of variation		Total flowers		Ovuliferous flowers			Ovules		Flower diameter		Stem height	
	df	MS	F	MS	F	df	MS	F	MS	F	MS	F
Sex	1	0.54	8.41**	13.46	155.5***	1	8.52	15.2***	5.98	380.1***	2.57	37.6***
Region	3	1.25	2.0	1.27	2.5†	3	5.17	3.7*	5.42	9.8**	17.19	3.0†
Sex × region	3	0.09	1.4	0.46	5.3**	3	0.25	0.4	0.10	6.12***	0.12	1.8
Populations (region)	22	0.75	11.8***	0.59	6.8***	10	1.38	2.5**	0.55	34.9***	5.66	82.8***
Error	2000	0.06		0.08		542	0.56		0.02		0.07	

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Fragaria virginiana (Ashman 1999) and *Daphne laureola* (Alonso and Herrera 2001). In *Hebe* and *Fragaria*, female frequency in populations was found to be negatively correlated with fruit set of hermaphrodites, indicating that females were more common in sites where their relative seed fitness is the highest (see also Wolfe and Shmida 1997, Delph and Carroll 2001). In *W. biglandulosa*, we found no evidence that female function of hermaphrodites was reduced in populations with more females. However, we examined ovuliferous flower production and it is possible that fruit and seed set are more environmentally labile (e.g. Delph 1990a).

Populations of *W. biglandulosa* are common in disturbed grassland and on granite outcrops. Plants occurred at high densities in both habitats, but plants on granite outcrops are smaller (G. Vaughton and M. Ramsey unpublished data), indicating that these habitats may be more stressful. Despite this we found no evidence that habitat influenced either the occurrence of gynodioecy or female frequencies in populations. In *W. dioica*, Case and Barrett (2001) demonstrated substantial ecological differentiation of hermaphroditic and dimorphic populations occurring in sympatry, including differences in plant density and flowering time. These differences resulted in the two sexual systems having different pollinators and potentially different patterns of mating. We are currently investigating whether such fine-scale ecological differentiation occurs in *W. biglandulosa* by focussing on gynodioecious and hermaphroditic populations that occur in close proximity in northern parts of the species' range.

Morphological and functional variation in gender of polliniferous individuals was similar in populations of both sexual systems. This variation ranged from plants that produced all perfect flowers, to those that produced perfect flowers in lower positions and male flowers in distal positions, and finally to small numbers of plants that produced male flowers only. In *W. biglandulosa*, polliniferous individuals are able to modify their gender and flower as either

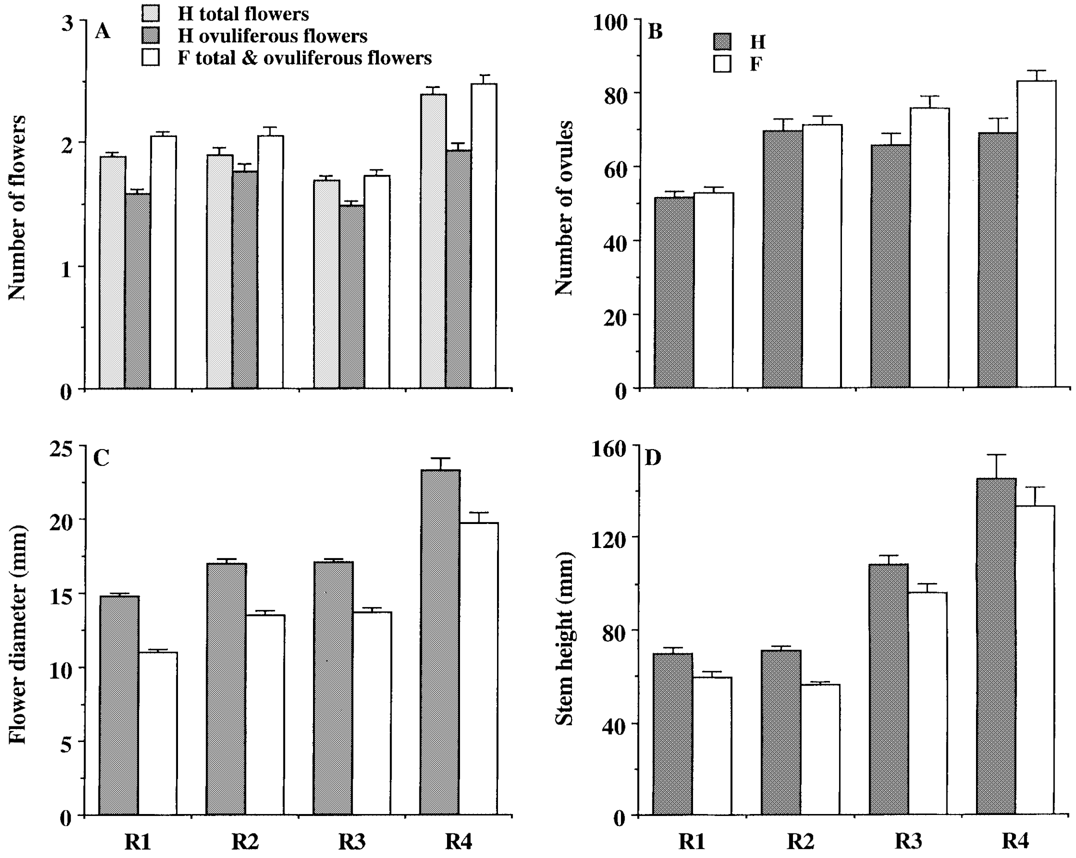

Fig. 3. Gender dimorphism between hermaphrodites and females in gynodioecious populations of *Wurmbea biglandulosa* grouped by geographic regions arranged from north (R1) to south (R4). The histograms represent the mean (± SE) number of total and ovuliferous flowers (**A**), the number of ovules per flower (**B**), flower diameter (**C**) and stem height (**D**) for each region. For (**A**), the number of total and ovuliferous flowers on females is equivalent

males or hermaphrodites. In this study, males were uniformly small and were probably lacking resources or flowering for the first time. However, when males are moved from the field to the glasshouse they increase in size, and flower in subsequent years as hermaphrodites. Similarly in the glasshouse and the field, larger hermaphrodites are more likely to produce all perfect flowers (G. Vaughton and M. Ramsey unpublished). These findings indicate the likely existence of size thresholds for the expression of female function (Delph and Lloyd 1991, Wolfe and Shmida 1997). Size-dependent gender modification also occurs in *W. dioica* (Barrett et al. 1999, Ramsey and Vaughton 2001), although the thresholds differ from those in *W. biglandulosa*. In *W. biglandulosa*, polliniferous individuals flower predominantly as hermaphrodites but in *W. dioica* such individuals flower predominantly as males.

Females can potentially compensate for an absence of male function by reallocating resources for pollen production into seeds (Eckhart 1992, Ashman 1994). Another possible source of resources for compensation in females is reduced investment in pollinator attractants if these are more important for male fitness than female fitness. In *W. biglandulosa*, although females produced marginally more flowers than hermaphrodites, individual flowers were smaller and stems were shorter, contributing to a smaller overall floral display on each female. Similar gender specialization occurs in *W. dioica* (Barrett et al. 1999, Ramsey and Vaughton 2001). Reduced allocation to pollinator attraction by females is common in temperate, animal-pollinated gynodioecious species and can be explained because male mating success is often limited by access to mates, whereas female success is often assumed to be limited by resources (Bell 1985, Delph et al. 1996). By producing larger floral displays hermaphrodites are likely to attract more pollinators, and thus increase their male fitness by dispersing more pollen (Vaughton and Ramsey 1998). Such male-biased selection on floral traits is expected to be greatest when pollinators are abundant and females are rare in populations (Ashman and Diefenderfer 2001).

No information is available concerning the inheritance of male sterility in *W. biglandulosa*. When sex determination is under nuclear-cytoplasmic control female frequencies are typically variable and can exceed 50% (Webb 1999). However, female frequencies in our study populations were generally low and none exceeded 50%, which is more consistent with nuclear control. When sex determination is nuclear, females require a two-fold advantage in seed fertility in order to establish in populations (Charlesworth and Charlesworth 1978). In *W. biglandulosa*, females on average produced 1.28 times more ovuliferous flowers plant^{-1} and 1.22 times more ovules flower^{-1} than did hermaphrodites. If gender differences in ovuliferous flower and ovule production are coupled with similar differences in fruit and/or seed set, then females would have only a 1.56 fitness advantage which is insufficient for them to establish in populations, assuming nuclear control of male sterility. One factor that could contribute to the maintenance of females is higher seed quality in females if hermaphrodites are self-compatible and exhibit inbreeding depression following selfing (Schultz and Ganders 1996, Sakai et al. 1997). At least some gynodioecious populations of *W. biglandulosa* are known to be self-compatible (Ramsey and Vaughton 2002). In addition, gender differences in adult survival (Van Damme and Van Delden 1984) or vegetative reproduction (Stevens and Van Damme 1988) could contribute to the maintenance of females in populations. We are currently examining these factors and the maintenance of females in *W. biglandulosa*.

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