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Research article

Dry environments promote the establishment of females in monomorphic populations of *Wurmbea biglandulosa* (Colchicaceae)

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Abstract. In flowering plants, the evolution of dimorphic breeding systems from monomorphic ancestors can be associated with dry environments. One hypothesis to explain this pattern is that seed fertility of hermaphrodites decreases more than seed fertility of females under dry conditions, so that females have greater relative fitness. This could occur if seed production of hermaphrodites is more resource-limited than that of females, or shifts in pollination increase levels of selfing and inbreeding depression in hermaphrodites. Here we assess the role of dry environments in promoting a female fitness advantage in *Wurmbea biglandulosa* by focusing on monomorphic and dimorphic populations that occur along a longitudinal gradient of decreasing rainfall. Dimorphic populations occurred in sites with higher temperatures, lower rainfall and lower soil moisture. Overall, females had greater seed fertility than did hermaphrodites from monomorphic populations, which in turn had greater seed fertility than hermaphrodites from dimorphic populations. Ovuliferous flower and ovule production by the three gender morphs and seed fertility of females and hermaphrodites in monomorphic populations did not vary with soil moisture. By contrast, seed fertility of hermaphrodites in dimorphic populations was positively related to soil moisture. Accordingly, female frequency was higher in those sites where hermaphrodites produced relatively fewer seeds. Taken together our results indicate that dry environments promote the establishment of females by decreasing the relative seed fitness of hermaphrodites. Moreover, because seed fertility of hermaphrodites in monomorphic populations did not vary with soil moisture, resource limitation of female function may play only a minor role in the establishment of females. Other factors such as shifts in pollination and mating patterns of hermaphrodites could be involved.

Key words: breeding system evolution, environmental stress, gender dimorphism, gynodioecy, sex ratio variation

Introduction

Almost all gender variation in flowering plants can be accommodated within two essentially distinct categories (Lloyd, 1980). Monomorphic populations contain individuals that on average contribute to the next generation equally as both male and female parents. By contrast, dimorphic populations show bimodality in gender because there are two distinct sexual morphs that function primarily as either males or females. For example, gynodioecy is a dimorphic sexual system in which females coexist with hermaphrodites in populations. Females do not produce pollen and achieve fitness via ovules only. Hermaphrodites in dimorphic populations achieve the majority of their fitness through pollen because they fertilize the ovules of females as well as the ovules of other hermaphrodites (Lloyd, 1976). The degree to which hermaphrodites function as males depends on the frequency of females. Hermaphrodites are expected to be more male in terms of their functional gender as females increase in frequency (Lloyd, 1976; Delph, 1990a).

Gynodioecy evolves when a male-sterile mutant invades and spreads in a population of hermaphrodites. For females to persist in frequencies greater than can be maintained by mutation alone, they must compensate for their loss of male fitness by producing either more or higher quality seeds than hermaphrodites. The magnitude of the seed advantage required depends on the mode of sex determination and is greater when nuclear genes rather than cytoplasmic genes control male sterility (Lewis, 1941; Charlesworth and Charlesworth, 1978; Charlesworth, 1981, 1999; Frank, 1989). Once females invade a population, their equilibrium frequency is influenced by the magnitude of their fitness advantage. Higher relative female fitness results in higher female frequencies, with the exact relationship being determined by the mode of sex determination. Moreover, high female frequency imposes selection on hermaphrodites to invest more resources in pollen and less in ovules, such that pure males may be favoured and the population becomes dioecious (Lloyd, 1976; Charlesworth, 1981, 1999; Delannay *et al.*, 1981; Frank, 1989; McCauley and Taylor, 1997).

Despite the broad significance of relative seed fitness in the evolution of gender dimorphism, the role of ecological factors in determining variation in seed fitness of the gender morphs has received comparatively little attention. Increasing evidence implicates environmental stress, particularly associated with dry habitats, in the evolution of gender dimorphism (reviewed by Sakai and Weller, 1999; Delph, 2003). Several studies have reported that gender dimorphism is more prevalent in drier environments (Hart, 1985; Barrett, 1992; Costich, 1995; Weller *et al.*, 1995; Case and Barrett, 2001; Vaughton and Ramsey, 2002), and that populations in drier or poorer sites have hermaphrodites with lower seed fertility and higher frequencies of females (Webb, 1979; Delph, 1990a; Wolfe and Shmida, 1997; Ashman, 1999; Alonso and Herrera, 2001; Delph and Carroll, 2001; Asikainen and Mutikainen, 2003; Case and Barrett, 2004a). Such patterns indicate that drier environments have a greater influence on seed fitness of hermaphrodites than females. This could come about if limited resources in drier environments reduce the ability of hermaphrodites to maintain female function, such that relative seed fitness of hermaphrodites is reduced as the availability of moisture declines (Delph, 1990a; Ashman, 1999; Ashman *et al.*, 2001; Delph, 2003). Alternatively, drier environments may affect pollination and mating patterns of plants, causing an increase in self fertilization and the expression or strength of inbreeding depression in hermaphrodites compared with females that are obligately outcrossed (Delph, 1990b; Delph and Lloyd, 1996; Schultz and Ganders, 1996; Sakai *et al.*, 1997; Case and Barrett, 2001, 2004a,b). Such gender-differential effects of drier environments could provide the necessary advantage required for females to invade and establish in hermaphroditic populations (Charlesworth, 1999).

Species or closely related taxa exhibiting variation in sexual systems provide valuable comparisons for studying ecological hypotheses proposed to explain the evolution of gender dimorphism (Delph, 1990b; Barrett, 1992; Costich, 1995; Weller *et al.*, 1995; Case and Barrett, 2001, 2004a). In such species, the transition between monomorphism and dimorphism is likely to have occurred relatively recently, and ecological differences between sexual systems are likely to have influenced selection for gender dimorphism. In an earlier broad-scale study, we reported intraspecific variation in sexual system and female frequency in *Wurmbea biglandulosa* ssp. *biglandulosa* (Vaughton and Ramsey, 2002). We found that dimorphic populations occurred in lower rainfall sites than monomorphic populations. However, female frequency was not correlated with either rainfall or female function of hermaphrodites, as assessed by the production of ovuliferous flowers.

Here we extend our previous study on *Wurmbea biglandulosa* by focusing on the northern part of the species' range where both sexual systems occur along a longitudinal gradient of decreasing rainfall. We first examine plants in 65 monomorphic and dimorphic populations to assess how gender expression varies along the rainfall gradient. We then characterize the environmental factors that might influence variation in gender expression by assessing site quality in a subset of these populations. Finally, we compare plant size, floral display and components of female function among the three gender morphs and determine whether female function is related to environmental factors. We predict that if dry conditions contribute to the maintenance of gender dimorphism, then variation in moisture availability should have a greater effect on female function of hermaphrodites than on females in dimorphic populations, providing a fitness advantage to females (Delph 1990a, 2003). Moreover, if environmental lability of female function has been important in the evolutionary transition to gender dimorphism, then we predict that hermaphrodites in monomorphic populations would also vary their female function in response to variation in moisture availability.

Methods

Study species and sites

Wurmbea biglandulosa (R. Br.) T.D. Macfarl ssp. *biglandulosa* is a diminutive winter-growing geophyte that grows in grassland or on granite outcrops in south eastern Australia (Macfarlane, 1980). Monomorphic populations are restricted to tableland sites in northern New South Wales and southern Queensland while dimorphic populations occur more widely in northern, central and southern New South Wales and north-eastern Victoria (Vaughton and Ramsey, 2002). The present study was conducted in northern New South Wales where our original survey detected both monomorphic and dimorphic populations located along a longitudinal rainfall gradient. In this area, rainfall is distributed throughout the year with a slight peak in summer.

Plants have a corm, a single unbranched stem, 2–3 annual leaves, and an inflorescence spike with 1–6 (usually 1–4) flowers. Vegetative reproduction occurs by the production of a single daughter corm. Flowering occurs in spring and flowers are insect-pollinated. The sex determination mechanism in *W. biglandulosa* is unknown. In both monomorphic and dimorphic populations, polliniferous plants produce varying proportions of staminate and perfect (hereafter ovuliferous) flowers. In dimorphic populations, females produce only pistillate (ovuliferous) flowers (Vaughton and Ramsey, 2002). We hereafter follow convention and refer to all polleniferous plants in dimorphic populations as males, and all plants in monomorphic populations as cosexuals (Lloyd and Bawa, 1980).

Sexual system variation

We assessed sex ratios in 65 populations and classified populations as either monomorphic ($n = 25$) or dimorphic ($n = 40$, Fig. 1). Populations were at least 1 km apart and were mapped with a hand-held global positioning system (GPS). The gender of at least 500 flowering plants or all plants if the population size was less than 500, was recorded while walking transects through populations. Plants were at least 0.5 m apart, ensuring that separate genotypes were sampled. Only plants with all their flowers open were scored.

Sex ratios were scored during peak flowering in 1999, 2000 and 2001. To assess whether sex ratios within populations varied, we correlated female frequencies between years in a subset of 15 populations. For these populations, mean values were used in subsequent analyses. The average number of plants examined in each population in each year was 642 ± 25 (range, 179–1243).

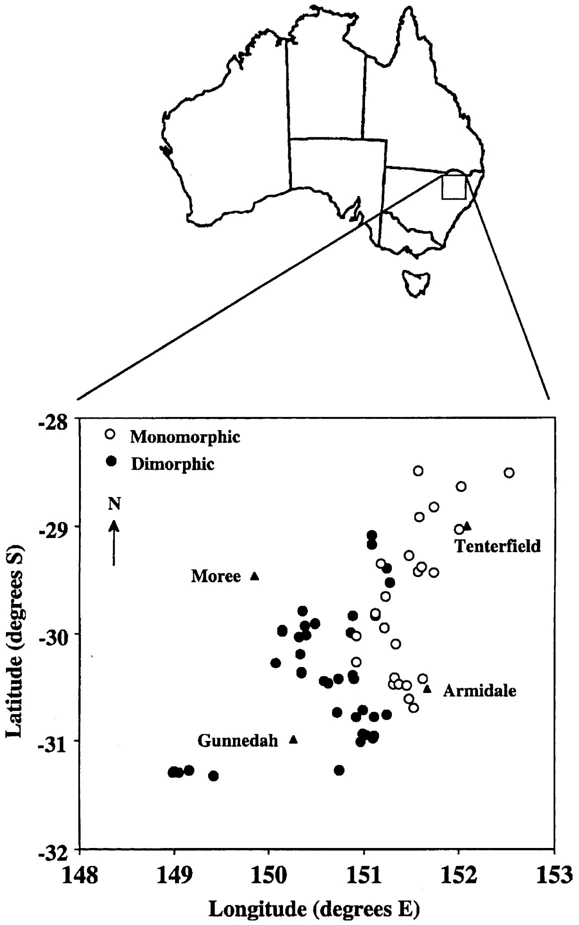

Figure 1. The location of 65 *Wurmbea biglandulosa* populations surveyed for sex ratios in northern New South Wales, Australia. Monomorphic populations (open circles) were located in the east and dimorphic populations (filled circles) were located in the west. Filled triangles represent local towns.

Climate

We estimated the climate for each of our sampled populations and developed a bioclimatic profile for monomorphic versus dimorphic populations using BIOCLIM, a component program of the software package ANUCLIM Version 5.1. BIOCLIM uses mathematical descriptions known as climatic surfaces to estimate climatic variables at user specified points within a region (Houlder *et al.*, 2001). For each site, BIOCLIM uses mean monthly maximum and minimum temperatures, rainfall, radiation and evaporation surfaces to derive up to 35 climatic parameters. We supplied BIOCLIM with GPS-derived latitude, longitude and altitude coordinates for each population and generated two output files: (1) for each population, the estimated values for the climatic parameters, and (2) the bioclimatic profile for each sexual system. Our sampling covered only a portion of the known range of *W. biglandulosa* (Vaughton and Ramsey, 2002) and the bioclimatic profiles pertain only to this area. Further details on the BIOCLIM methodology are given in Lindenmayer *et al.* (1991) and Fisher *et al.* (2001).

Many of the climatic parameters generated by BIOCLIM are correlated and the resultant bioclimatic profile does not directly indicate which parameters have the greatest effect on distribution. To define more precisely the effect of climate on sexual systems we used principal component analysis (PCA). A data set was constructed and consisted of all sites and values for 16 climatic parameters for each site (Table 1). These 16 parameters were chosen from the full set of 35 parameters to provide an approximately equal weighing to temperature, rainfall, radiation and moisture indices, and on the basis of bimodal responses for monomorphic and dimorphic populations as indicated by diagnostic plots provided by BIOCLIM. For the PCA, data were reduced to principal components with eigenvalues greater than 1.0 and unrotated scores were used. We compared the first principal component (PC1) scores for the two sexual systems with one-way ANOVA.

Table 1. Mean and range (in parentheses) of 16 climate parameters calculated for monomorphic ($n = 25$) and dimorphic ($n = 40$) populations of *Wurmbea biglandulosa*. CV is coefficient of variation expressed as a percentage

Description of bioclimatic index	Monomorphic	Dimorphic
Temperature		
Mean annual, °C	14.3 (11.7–16.9)	15.4 (12.4–17.5)
Mean diurnal range, °C	13.4 (12.1–14.7)	14.6 (13.6–15.7)
Seasonality, CV	1.72 (1.46–1.86)	1.91 (1.78–2.02)
Annual range, °C	27.1 (24.5–29.6)	29.8 (27.7–31.6)
Max. of warmest period, °C	27.7 (24.7–30.5)	30.4 (26.7–33.1)
Precipitation		
Mean annual, mm	783 (670–1029)	750 (637–930)
Seasonality, CV	32 (28–45)	28 (18–34)
Mean of wettest quarter, mm	285 (254–420)	260 (214–309)
Mean of warmest quarter, mm	278 (245–411)	256 (213–309)
Radiation		
Highest period radiation	27.7 (23.8–25.3)	25.3 (24.2–26.2)
Lowest period radiation	10.3 (9.9–10.8)	9.8 (8.8–10.5)
Seasonality, CV	27 (25–28)	29 (27–32)
Moisture Index		
Annual mean	0.54 (0.40–0.69)	0.27 (0.20–0.35)
Seasonality, CV	24 (17–31)	33 (21–44)
Mean of low quarter	0.40 (0.31–0.50)	0.33 (0.26–0.43)
Mean of warm quarter	0.49 (0.39–0.69)	0.37 (0.27–0.47)

Microhabitat variation

The BIOCLIM analysis provides data to test for large-scale patterns of association between climate and sexual systems. However, habitat factors may operate at a micro-environmental scale to influence sexual systems. Accordingly, we examined site quality in a subset of 14 monomorphic and 26 dimorphic populations by measuring soil depth, soil moisture, and vegetative cover. Sampling was undertaken during the flowering season in 2002.

Soil depth was determined by hammering a 30 cm long metal probe as far as possible into the soil at 20 locations at each site. Measurements were taken within 2 cm of a *W. biglandulosa* plant. Soil moisture was examined by extracting 10 soil cores from each site. Soil cores (3 cm diameter) were taken within 5 cm of a *W. biglandulosa* plant and were to a depth of 10 cm, the maximum depth of corms in these sites. Leaf litter was not included in the sample. No rain had fallen at any of the sites for at least two weeks prior to sampling. Soil was placed in airtight tubes and weighed within 4 h to the nearest 0.1 mg. Samples were reweighed after drying to a constant weight at 60 °C for 7 days in a drying oven. Soil moisture content was calculated gravimetrically as: (wet mass – dry mass) / dry mass.

Vegetative cover was assessed by running ten, 5 m transects through each population and determining whether 25 points (one every 20 cm) on each transect intercepted rock, bare soil or plant material. Plant material included both green and standing dead material. Vegetative cover was the proportion of points intercepting plant material and was interpreted as the capacity of each site to support plant growth.

We used nested ANOVAs to examine differences in soil moisture, vegetative cover and soil depth between sexual systems. Populations were considered random factors and were nested within sexual system, a fixed factor.

Plant size, floral display and female function

We assessed plants in 14 monomorphic and 12 dimorphic populations. In each population, we recorded flower number, flower diameter and stem height on 20 plants of each gender. Plants were selected by laying-out transects and choosing flowering plants that were at least 1 m apart. For cosexuals and males, we assessed the total number of flowers and the number of these that were ovuliferous. Females produce only ovuliferous flowers. Flower diameter was measured as the distance between the tips of two opposite tepals on the first flower; the mean of two measurements was used in analyses. Stem height was measured as the distance from the soil to the base of the first flower.

We assessed fruit set by marking at least 50 flowering plants of each gender in each population. We included polliniferous only plants in our sample of males and cosexuals in proportion to their frequency in populations (i.e., 5%, Vaughton and Ramsey, 2002). The proportion of plants producing fruits in each population was scored 6 weeks later. Fruiting plants produced at least one fruit. For each gender we harvested a subset of 20 fruiting plants in each population to assess the numbers of ovules per flower, seeds per fruit and seeds per fruiting plant. We counted ovules by dissecting ovaries of the first fruit on inflorescences. The number of ovules was the sum of the number of unfertilized ovules, aborted seeds and viable seeds. We were unable to assess either fruit or seed production of females in one population. In two other populations only 12 and 17 females were assessed.

We tested for differences in plant height, number and size of flowers, and seed fertility components among the three gender morphs using nested ANOVAs with gender as a fixed factor and population as a random factor nested within gender. For the proportion of plants setting fruit and average seed fertility per plant, there was no replication at the population level, and we used one-way ANOVAs to assess gender differences. We estimated average seed fertility as the proportion of plants setting fruit × the mean number of seeds per fruiting plant. For all analyses, we employed non-orthogonal, planned contrasts to test for differences between means of the gender morphs. To reduce type one errors in these tests, we used a sequential Bonferroni method to adjust α (Rice, 1989).

We used ANCOVAs to determine the effect of environmental variation on pre- and post-fertilization components of female function using population means for ovuliferous flower production, ovule production per flower and average seed fertility per plant. We used soil moisture as an environmental covariate, because in preliminary analyses other environmental variables (PC1, PC2 from the BIOCLIM analysis, vegetative cover) showed no association with any of the components of female function. For each component, we assessed the significance of the covariate × gender interaction in the ANCOVA. A significant interaction indicates that the effect of soil moisture differs between the gender morphs. If significant, we then examined the relationship between soil moisture and the component separately for each gender using least-squares linear regression. If the interaction was not significant, then we removed it from the model and tested whether the soil moisture covariate was significant. A significant covariate effect indicates that the component of female function is related to soil moisture.

Relative hermaphrodite seed fitness and female frequency

We estimated relative seed fitness of males as the ratio of male to female seed fertility for each dimorphic population. For monomorphic populations, we estimated relative seed fitness of cosexuals as the ratio of cosexual seed fertility to the overall mean female seed fertility of all dimorphic populations. To determine whether females were more common in sites where males produced fewer seeds, we correlated female frequency with relative male seed fertility. We also assessed whether female frequency and male and cosexual relative fitnesses were related to soil moisture using linear regressions.

Statistics

To meet assumptions of normality and homogeneity of variances, plant height, flower diameter, and numbers of seeds per fruiting plant and per plant were transformed using natural logarithms. Numbers of seeds per fruit were square-root transformed. Means ± SE are presented. Analyses were performed using JMP (version 5.01a, SAS Institute, 2002).

Results

Sexual system variation

In dimorphic populations, female frequencies varied from 3 to 54%, with a mean of 16% ± 2. By contrast, monomorphic populations contained on average 0.25% ± 0.06 females (range, 0.0 – 0.8%). Little variation in population sex ratios occurred among years. Female frequency in the five monomorphic populations that were assessed in two or more years varied by less than 0.5% among years. Similarly, female frequencies in 10 dimorphic populations varied by an average of only of 4.8% ± 0.9 among years. Using all data, female frequencies were strongly correlated between different years (Pearson correlation, $r = +0.96$, df = 13, $p < 0.001$).

The sexual systems were geographically segregated. Dimorphic populations occurred at lower elevations in more western inland areas than monomorphic populations (Fig. 1; elevation: 609 ± 30 m vs. 819 ± 37 m, $F_{1,63} = 19.22$, $p < 0.001$; longitude: 150.6 ± 0.1 °E vs. 151.5 ± 0.1 °E, $F_{1,63} = 45.30$, $p < 0.001$). A 40 km wide area of overlap where both sexual systems co-occurred was present in the center of the study area (Fig. 1).

Environmental correlates of sexual system variation

Differences between sexual systems for the 16 climatic parameters are shown in Table 1. Data reduction by PCA produced three principal components that accounted for 94% of the variation in the climatic variables (PC1 = 67%, PC2 = 17%, PC3 = 10%). PC1 was positively correlated with temperature and radiation, and negatively correlated with rainfall and moisture indices.

All sites were plotted using their scores for PC1 and PC2 (Fig. 2). The sexual systems formed two clusters that were separated by their PC1 scores. Dimorphic populations had positive PC1 scores and monomorphic populations had negative scores (1.8 ± 0.4 vs. −2.8 ± 0.5, $F_{1,63} = 54.73$, $p < 0.001$). This indicates that dimorphic populations were located in sites with higher temperatures and radiation but lower rainfall and less favourable moisture indices than were monomorphic populations.

Compared with monomorphic populations, dimorphic populations had significantly lower soil moisture (g water/g soil: 0.195 ± 0.008 vs. 0.084 ± 0.004, $F_{1,38} = 23.35$, $p < 0.001$) and less vegetative cover (percent cover: 81.1 ± 1.5 vs. 66.4 ± 1.4, $F_{1,38} = 6.31$, $p < 0.05$). Soil depth, however, did not differ between sexual systems (22.3 ± 0.5 cm vs. 20.8 ± 0.4 cm, $F_{1,38} = 0.88$, $p > 0.80$), and was omitted from further analyses. Significant variation occurred among populations in all three analyses (all $p < 0.001$).

Plant size, floral display and seed fertility

Stem height differed significantly among the gender morphs ($F_{2,35} = 10.68$, $p < 0.001$; cosexuals: 92.0 mm ± 2.0, males: 70.9 mm ± 1.5, females: 58.8 mm ± 1.4). Cosexuals were taller than both females ($p < 0.001$) and males ($p < 0.015$, Bonferroni $\alpha = 0.025$), and males tended to be taller than females ($p = 0.055$, $\alpha = 0.05$). Flower diameter also differed significantly ($F_{2,35} = 39.71$, $p < 0.001$; cosexuals: 17.3 mm ± 0.2, males: 15.7 mm ± 0.2, females: 12.2 mm ± 0.1). Females produced smaller flowers than both males and cosexuals (both $p < 0.001$), and males had smaller flowers than cosexuals ($p = 0.024$, $\alpha = 0.05$). For both traits, significant variation occurred among populations (both $p < 0.001$).

The gender morphs produced similar numbers of total flowers, but females produced more ovuliferous flowers than did both males and cosexuals, which did not differ (Table 2, Fig. 3a). On average, 88% of flowers on cosexuals and 84% of flowers on males were ovuliferous. The number of plants producing fruits differed significantly ($F_{2,34} = 17.33$, $p < 0.001$), and fewer males pro-

Table 2. *F*-values from nested ANOVAs showing the effects of gender and population nested within gender on components of female function for cosexuals in monomorphic populations and males and females in dimorphic populations. Analyses refer to data in Fig. 3

Source of variation	df	Total flowers	Ovuliferous flowers	df	Ovules per flower	Seeds per fruit	Seeds per fruiting plant
Gender	2,35	0.24	7.89^{**}	2,34	0.71	3.67^{*}	6.73^{**}
Population (gender)	34,719	4.46^{***}	3.86^{***}	34,692	10.08^{***}	5.76^{***}	6.51^{***}

$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

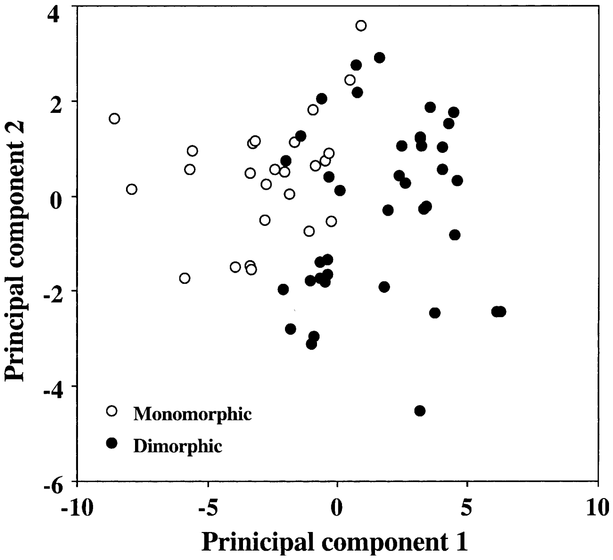

Figure 2. Principal components analysis of 16 climatic parameters for monomorphic (open circles) and dimorphic (filled circles) populations of *Wurmbea biglandulosa*. Sites are plotted against the first two principal components, which accounted for 67 and 17% of the variation in the data set, respectively.

duced fruits than did either females or cosexuals, which did not differ (Fig. 3b). The three gender morphs produced similar numbers of ovules per flower (cosexuals: 65.8 ± 0.9, males: 62.3 ± 1.1, females: 65.1 ± 1.03, Table 2). By contrast, the numbers of seeds produced per fruit differed significantly. Females produced about five seeds and two seeds per fruit more than did males and cosexuals, respectively, although the latter was not significant (Table 2, Fig. 3c). Seeds per fruiting plant also differed significantly. Females produced about 21 and 14 more seeds per plant than did males and cosexuals, respectively. This latter difference neared significance ($p = 0.037$, $\alpha = 0.025$; Table 2, Fig. 3d). Significant variation occurred among populations in all analyses (Table 2). Average seed fertility per plant also differed significantly ($F_{2,34} = 9.20$, $p < 0.001$; cosexuals: 31.7 ± 3.4, males: 21.8 ± 3.1, females: 45.7 ± 7.1). Females produced more seeds than males ($p < 0.001$) and cosexuals ($p < 0.047$, $\alpha = 0.050$), and cosexuals produced more seeds than males ($p = 0.020$, $\alpha = 0.025$).

In ANCOVAs to examine the effects of soil moisture on the numbers of ovuliferous flowers per plant and ovules per flower, the gender × soil moisture (covariate) interactions were not significant (flowers: $F_{2,32} = 0.30$, $p > 0.70$; ovules: $F_{2,31} = 0.99$, $p > 0.35$). When the interactions were removed from analyses, soil moisture was not significant, indicating that pre-fertilization components of female function were not related with variation in soil moisture

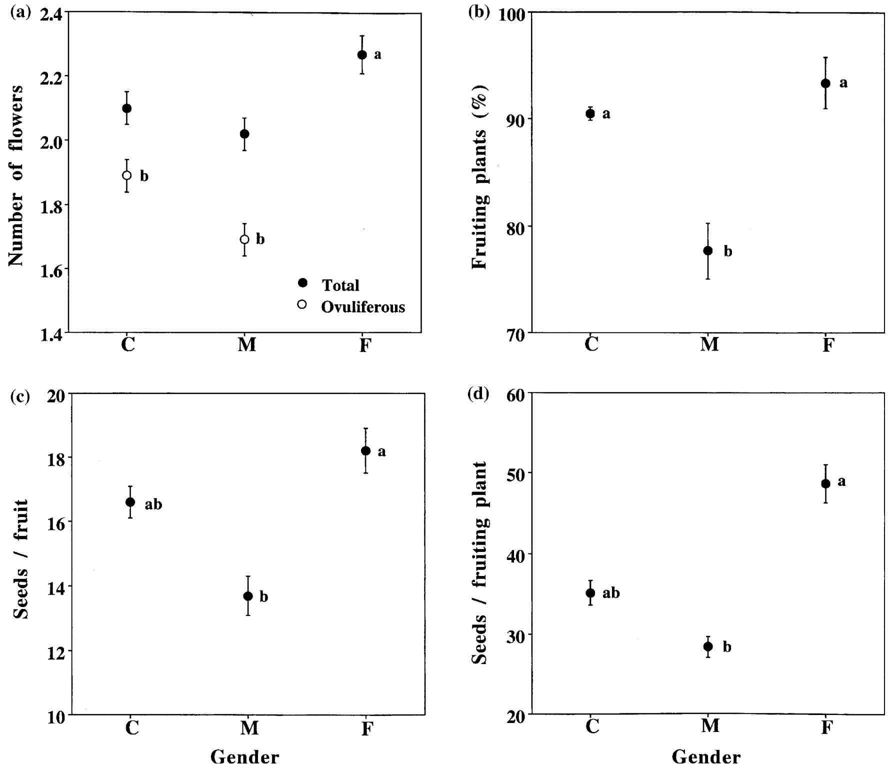

Figure 3. Mean (± SE) numbers of flowers (a), percentage of plants producing fruit (b), numbers of seeds per fruit (c), and seeds per fruiting plant (d) for *Wurmbea biglandulosa.* Data are for cosexuals in monomorphic populations and males and females in dimorphic populations. Means with different letters differed significantly (planned contrasts using adjusted α from sequential Bonferroni tests). Total number of flowers did not differ among gender morphs, but females produced more ovuliferous flowers than did cosexuals and males.

(flowers: $F_{1,34} = 0.26$, $p > 0.60$; ovules: $F_{1,33} = 0.10$, $p > 0.70$). By contrast, the gender × soil moisture interaction for average seed fertility was significant ($F_{2,31} = 3.47$, $p = 0.044$). For males, seed fertility was positively related to soil moisture, indicating that males produced more seeds in wetter sites and fewer seeds in drier sites (Fig. 4). For cosexuals and females, seed fertility was unrelated to soil moisture (Fig. 4). Female seed fertility remained unrelated to soil moisture when we omitted two outlying values at low soil moistures ($p = 0.115$).

Female frequency and relative male seed fitness

In dimorphic populations, female frequency was negatively correlated to relative male seed fitness (Fig. 5), but was not related to soil moisture

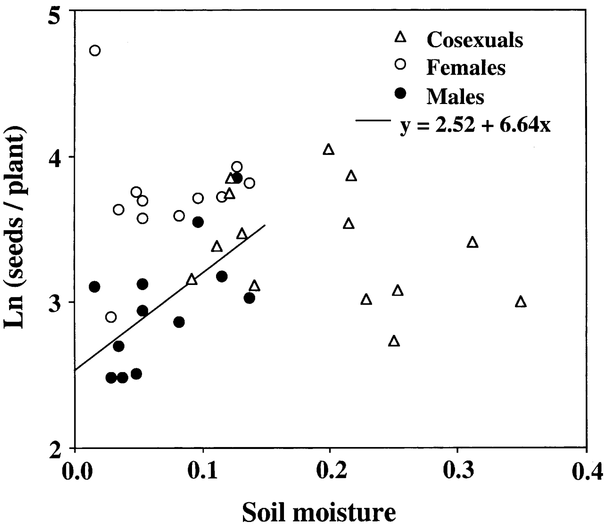

Figure 4. Relationships between soil moisture (g water/g dry soil) and numbers of seeds per plant (ln transformed) produced by cosexuals from monomorphic populations, and females and males from dimorphic populations of *Wurmbea biglandulosa*. Least squares regression was significant for males ($F_{1,10} = 7.30$, $p = 0.022$, $r^2 = 0.42$), but not for females ($F_{1,9} = 0.00$, $p > 0.90$) or cosexuals ($F_{1,12} = 1.49$, $p > 0.20$).

($F_{1,24} = 1.85$, $p > 0.18$). In all of these populations, relative seed fitness of males was less than 1.0, averaging 0.54 ± 0.07 (range, 0.20–0.93), and was positively related to soil moisture ($F_{1,9} = 5.10$, $p = 0.050$, $r^2 = 0.36$). By comparison, the relative seed fitness of cosexuals was 0.70 ± 0.07 (range, 0.44 – 1.25). Relative cosexual fitness was not related to soil moisture ($F_{1,12} = 1.06$, $p > 0.30$).

Discussion

Our aim in this study was to assess the role of dry environmental conditions in the evolution of gynodioecy in *Wurmbea biglandulosa* by focusing on monomorphic and dimorphic populations occurring along a longitudinal gradient of decreasing rainfall. We found that dimorphic populations occurred on soils with lower moisture content, and in areas with lower rainfall and higher temperatures than monomorphic populations. Moreover, seed fertility of males was more variable in response to variation in soil moisture than was the case for either coexisting females or cosexuals from monomorphic populations. Among dimorphic populations, females were more common in sites where males produced relatively fewer seeds, although no relationship existed between soil moisture and female frequency. Taken together our results

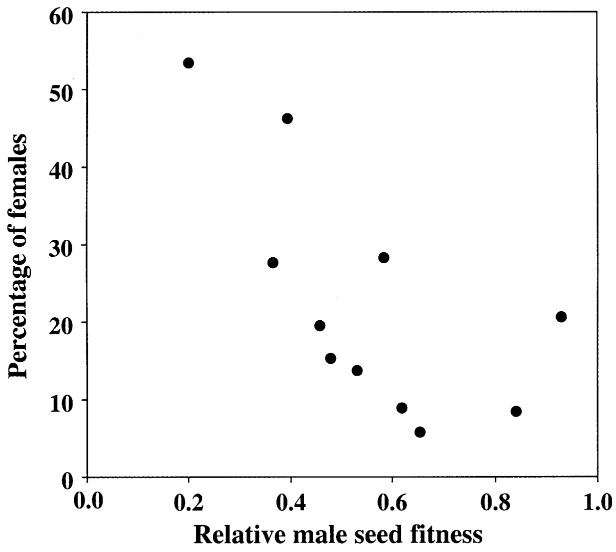

Figure 5. Negative relationship between relative male seed fitness and female frequency in 11 dimorphic populations of *Wurmbea biglandulosa* ($r = -0.69$, df $= 9$, $t = 2.89$, $p = 0.018$).

indicate that drier environments decrease male fitness relative to female fitness and can, therefore, promote the establishment of females in monomorphic populations of *W. biglandulosa*. Below we consider the spatial segregation of sexual systems and discuss how differences between the gender morphs in their response to variation in soil moisture may influence the establishment of females in monomorphic populations.

Monomorphic and dimorphic sexual systems of *W. biglandulosa* were spatially segregated into different types of habitats. Dimorphic populations occurred at lower elevations and experienced hotter and drier climatic conditions than monomorphic populations, indicating a broad-scale association between dry conditions and gender dimorphism. The climatic differences between sexual systems that were identified in the BIOCLIM analysis concur with previous findings based on rainfall data from local weather stations throughout the range of *W. biglandulosa* (Vaughton and Ramsey, 2002). Gender dimorphism was also more prevalent in sites with low soil moisture and less vegetation cover, indicating an association between sexual system and plant growth conditions at a local scale. When both sexual systems occurred in close proximity and experienced similar climatic conditions, monomorphic populations occurred in wetter microsites, whereas dimorphic populations occurred in drier microsites. An association between gender dimorphism and drier soil conditions has also been reported in *W. dioica*. In this species, differences in plant size, physiology and biomass allocation patterns are associated with local-scale variation in water availability

(Barrett, 1992; Case and Barrett, 2001, 2004a). Spatial segregation of sexual systems also occurs in *Ecballium elaterium* with monoecious subspecies occurring in the wetter northern region of the Iberian Peninsula while dioecious subspecies occur only in drier areas of the south (Costich, 1995). Similarly, in Hawaiian *Schiedea*, dimorphic species are found in dry habitats, whereas monomorphic species occur in wet habitats (Weller *et al.*, 1995). These findings point to the need to better understand how dry environments affect relative female fitness and thereby ease the evolutionary transition from monomorphism to dimorphism.

We predicted that if dry conditions contribute to a female seed fitness advantage in *W. biglandulosa*, then variation in soil moisture should have a greater effect on female function of males than on females in dimorphic populations (Delph, 1990a, 2003). As predicted, seed fertility of males but not females declined along a gradient of decreasing soil moisture among dimorphic populations. The greater variation in seed fertility of males compared with females in response to dry conditions would help to maintain females in populations by increasing their relative seed fitness advantage. Studies of other gynodioecious species have reported similar declines in seed fertility of males with decreasing site quality (Delph, 1990a; Wolfe and Shmida, 1997; Ashman 1999). Such effects are often attributed to resource limitation in poor sites and the inability of males to maintain both sexual functions under such conditions. Because males achieve more fitness through pollen donation in dimorphic populations, they are expected to favour reductions in female function rather than male function as resources become limiting. By contrast, females have overall lower reproductive costs than seed-producing males and are less affected by resource limitation. Moreover, the presence of females in dimorphic populations increases the pollen fitness of males and may select for males with higher pollen production and lower seed production (Lloyd, 1976; Charlesworth, 1999).

In contrast to males, seed fertility of cosexuals was not related to variation in soil moisture occurring among monomorphic populations. This implies that lability of female function evolved after the establishment of females in populations. However, one problem with this conclusion is that cosexuals in monomorphic populations did not experience the driest sites, and therefore they may not have been as resource-limited as males in dimorphic populations. Where monomorphic and dimorphic populations occurred in soils with similar moisture content, cosexuals and males produced similar numbers of seeds (Fig. 4). Case and Barrett (2004a) used paired populations with contrasting sexual systems in areas of similar rainfall to examine lability of female function in *W. dioica*. They found that males but not cosexuals adjusted ovuliferous flower production in response to declining rainfall, supporting the view that although lability of female function was a factor maintaining females in dimorphic populations, it was unlikely to have been responsible for their initial establishment. Studies of monomorphic and dimorphic species of New Zealand *Hebe* support a similar conclusion (Delph, 1990a).

Plants can serially adjust their investment in female function to match available resources by altering flower production, ovary development, and maturation of fruits and seeds (Lloyd, 1980). In dimorphic populations of *W. biglandulosa*, males did not adjust numbers of ovuliferous flowers or ovules in response to variation in soil moisture, but instead adjusted fruit and seed set. By contrast in *W. dioica*, males adjust ovuliferous flower production (Barrett *et al.*, 1999; Ramsey and Vaughton, 2001; Case and Barrett, 2004a). Given the simple inflorescence structure in *Wurmbea* and the greater likelihood of basal flowers setting fruit, adjustment of female function at flower initiation would be less wasteful of resources than adjustment at the later stages of fruit and seed development (Barrett *et al.*, 1999). In *W. biglandulosa*, the less efficient mechanism by which female function is adjusted may be a consequence of this species being closer to the 'gynodioecious end' of the gender spectrum between gynodioecy and dioecy, and that males in dimorphic populations still produce substantial numbers of seeds. In other dimorphic species, female function is commonly adjusted by altering the percentage of flowers that are matured into fruit, indicating that perhaps control at flowering is difficult to evolve and may be related life-history (Delph, 1990a; Wolfe and Shmida, 1997; Ashman, 1999; Delph and Carroll, 2001).

Initial female establishment and their equilibrium frequency is predicted to vary, depending on the relative seed fitness of the gender morphs (Lloyd, 1976; Charlesworth, 1981). In *W. biglandulosa*, females may not have the required fitness advantage to become established in monomorphic populations. Cosexual relative seed fitness was on average was 0.70, substantially greater than 0.50, the value predicted by theory for the initial establishment of females, assuming nuclear control of sex determination (Charlesworth and Charlesworth, 1978). By contrast, the average relative male fitness in dimorphic populations was about 0.50, which is consistent with the presence of females. In these populations, male fitness decreased with decreasing soil moisture, which in turn explains the negative relationship between relative male fitness and female frequency. Interestingly, however, we failed to find a negative relationship between female frequency and soil moisture. One explanation for this finding is that female frequency is indirectly and only weakly related to site quality via relative male fitness. Negative relationships between female frequency and site quality have been demonstrated in other studies (e.g. *Hebe strictissima*, Delph, 1990a; *Fragaria virginiana* , Ashman, 1999; *Daphne laureola*, Alonso and Herrera 2001; *Geranium sylvaticum*, Asikainen and Mutikainen, 2003; *Wurmbea dioica*, Case and Barrett, 2004a).

In dry environments a seed fitness advantage may also accrue to females if low soil moisture influences plant mating systems, causing an increase in selfing or the expression of inbreeding depression (Delph, 1990b; Delph and Lloyd, 1996; Schultz and Ganders, 1996; Sakai *et al*., 1997; Case and Barrett, 2001, 2004a,b). Case and Barrett (2004a, b) proposed that shifts in mating systems are likely to have played a key role in the evolutionary transition from monomorphism to dimorphism in *W. dioica*. More specifically, they suggested that smaller flower size in drier habitats altered the effectiveness of nectar-foraging floral visitors, thereby increasing selfing in hermaphrodites. We also found that flowers were smaller under drier conditions in dimorphic populations of *W. biglandulosa*. Unlike plants in southern populations that are partially self-fertile (Ramsey and Vaughton, 2002), plants in northern populations are largely self-infertile, experiencing substantial seed abortion following self-pollination (M. Ramsey and G. Vaughton, unpublished data). In this study, lower fruit and seed set in males compared with females, despite similar ovule production, may have resulted from high levels of self-fertilization and subsequent abortion. To examine this, we are currently comparing pollination and selfing rates of the gender morphs in monomorphic and dimorphic populations.

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