



Research article

Environmental stress and the evolution of dioecy: *Wurmbea dioica* (Colchicaceae) in Western Australia

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Abstract. Stressful ecological conditions have been implicated in the evolution of separate sexes in plants. Gender dimorphic species are often found in drier habitats than their sexually monomorphic relatives, and gynodioecious populations appear closer to a dioecious state as resources, particularly water, become limiting. This pattern could result if dry conditions decrease the relative seed fitness of cosexual plants, allowing female plants to become established in monomorphic populations. We studied geographical variation in gender expression and biomass allocation among 12 monomorphic and dimorphic populations of *Wurmbea dioica* along a latitudinal precipitation gradient in southwestern Australia to provide insight into mechanisms by which aridity might favor transitions between sexual systems. Plants in monomorphic and dimorphic populations exhibited contrasting gender expression and patterns of biomass allocation in areas with different levels of precipitation. Among dimorphic populations, lower precipitation was associated with a higher frequency of female plants, and reduced allocation to female function by hermaphrodites during flowering. In contrast, stress conditions had no effect on female allocation at flowering in monomorphic populations. Across latitudes, unisexuals and cosexuals exhibited consistent differences in above ground traits, with cosexuals having larger leaves, taller stems and larger flowers. Although all plants were smaller under drier conditions, cosexuals decreased above ground allocation to vegetative and reproductive structures with decreasing latitude. In contrast, unisexuals increased allocation to reproduction in drier areas at the expense of below ground size. Aridity was associated with reduced flower size among all gender classes, but not with changes in flower number. These data do not support the hypothesis that resource limitation of female allocation in cosexual populations favors the establishment of gender dimorphism in *W. dioica*. Alternative hypotheses, involving higher selfing rates and enhanced survival of unisexuals relative to cosexuals under resource-limited conditions, are discussed as possible explanations for the origin of dioecy in *W. dioica*.

Key words: biomass allocation, environmental stress, evolution of dioecy, gender strategies, sex ratio

Introduction

The origin of separate sexes (dioecy) from combined sexes (cosexuality) via the gynodioecy pathway has occurred repeatedly during the evolutionary history

of the flowering plants (Webb, 1999; Weiblen *et al.*, 2000), and consists of two successive stages. The first involves the invasion of hermaphrodite (hereafter 'cosexual') populations by female plants, resulting in the dimorphic condition known as gynodioecy (Darwin, 1877). The second step occurs as hermaphrodites in gynodioecious populations (hereafter 'males') increasingly favor pollen over seed production (Lloyd, 1976) resulting in their replacement by pure males and the establishment of dioecy. The genetical aspects of this evolutionary transition are now well understood as a result of considerable theoretical and empirical research (reviewed in Charlesworth, 1999). However, less is known about the ecological mechanisms that favor the evolution of separate sexes from combined sexes via the gynodioecy pathway.

Several recent investigations have implicated stressful ecological conditions, particularly aridity, in the evolution of dioecy via the gynodioecy pathway (reviewed in Sakai and Weller, 1999; Delph, 2003). Empirical studies report a greater incidence of gender dimorphism (Hart, 1985; Weller and Sakai, 1990; Costich, 1995; Alonso and Herrera, 2001; Case and Barrett, 2001; Delph and Carroll, 2001; Vaughton and Ramsey, 2002) and increased gender specialization (Webb, 1979; Arroyo and Squeo, 1990; Delph, 1990a; Barrett, 1992; Costich, 1995; Wolfe and Shmida, 1997; Ashman, 1999; Asikainen and Mutikainen, 2003; Ramula and Mutikainen, 2003) in resource-limited habitats. Although these findings imply that resource limitation is involved in both stages of the evolutionary transition to dioecy, the mechanism by which stress favors gender specialization is more clearly understood. In several gynodioecious species, populations in more stressful habitats appear closer to dioecy, with higher frequencies of female plants and reduced relative seed fitness of males (Webb, 1979; Arroyo and Squeo, 1990; Delph, 1990a; Barrett, 1992; Wolfe and Shmida, 1997; Ashman, 1999; Asikainen and Mutikainen, 2003). Fruit production in hermaphrodites but not females is strongly affected by plant size, such that their female fertility is reduced as resource availability declines (Webb, 1979; Delph, 1990b; Delph and Lloyd, 1991; Wolfe and Shmida, 1997; Ashman, 1999; Barrett *et al.*, 1999). Here, we investigated whether resource limitation, particularly aridity, may favor the transition between gender monomorphism and gender dimorphism. We focused simultaneously on both the first and second stages of the gynodioecy pathway, comparing the responses of traits related to seed fitness along a latitudinal gradient in precipitation. Responses of cosexuals and females are relevant to stage 1, and those of females and males are relevant to stage 2.

For transitions between monomorphic and dimorphic sexual systems (i.e., stage 1) to be favored by low resource availability, the seed fitness of females relative to cosexual plants must be greatest in harsh habitats (Charlesworth and Charlesworth, 1987; Delph, 1990b; Sakai *et al.*, 1997; Delph and Carroll, 2001). There are three ways this could occur. First, if investment into ovules

increases with overall plant vigor in cosexuals but not females, then low resource availability will amplify the benefits of reproductive compensation for females (Delph, 1990b; Eckhart, 1992; Klinkhamer *et al.*, 1994; Costich, 1995). This is analogous to the argument invoked to explain the role of stress in the gynodioecy-dioecy transition (i.e., stage 2). Second, harsher conditions could increase the expression and/or strength of inbreeding depression in cosexuals, amplifying the outcrossing advantage for female plants (Charlesworth and Charlesworth, 1987; Delph, 1990a; Weller and Sakai, 1990; Weller *et al.*, 1990; Delph and Lloyd, 1996; Schultze and Ganders, 1996; Sakai *et al.*, 1997). Stress may influence the mating system either directly through changes in plant phenotype (e.g., flower size and number) or indirectly through effects on pollen vectors (e.g., shifts in pollinator activity or type). Third, the allocation patterns or physiology of females may enhance their growth and survival in low resource environments, which may lead to increases in lifetime seed production over cosexuals. This could result from pleiotropic effects of male-sterility loci (Lloyd, 1975), selection on females to enhance resource acquisition (Dawson and Geber, 1999), or may be a consequence of resource compensation.

An evaluation of the relative importance of these hypotheses requires the comparison of plants in monomorphic and dimorphic sexual systems across a range of resource conditions. Of particular significance in such a comparison is the effect of low resources on traits that contribute to relative seed fitness – investment in ovules, plant size and biomass allocation, and characters influencing the mating system, such as flower size and number. Closely related taxa with contrasting sexual systems are most appropriate for investigating this problem because the transition between monomorphism and dimorphism (stage 1) has occurred relatively recently and plants are likely to be similar in other aspects of ecology and morphology. Populations of gynodioecious species are less appropriate for testing hypotheses concerning the first step in the pathway. Although polleniferous plants in monomorphic and dimorphic populations can both be phenotypically hermaphroditic, they differ functionally in the manner in which they gain fitness, and would therefore be expected to respond differently to resource availability (Lloyd, 1976; Lloyd and Bawa, 1984; Webb, 1999). Because hermaphrodites in gynodioecious populations gain most of their fitness through pollen, they can exhibit greater lability of seed production than cosexuals, which on average gain fitness equally through pollen and seed. Therefore, an appropriate test of whether low resource availability facilitates the invasion of female plants into populations of cosexuals (stage 1) necessitates decreased female fertility by cosexuals (not by males) with changes in resource availability.

We investigated geographical variation in gender expression and biomass allocation in monomorphic and dimorphic populations of *Wurmbea dioica* (R.

Br.) *F. Muell ssp. alba* Macfarlane (Colchicaceae) along a latitudinal precipitation gradient in southwestern Australia. *Wurmbea dioica ssp. alba* is a diminutive, self-compatible, insect-pollinated geophyte that is common throughout southwestern Australia. Population surveys indicate considerable variation in sexual systems, ranging from monomorphic populations to gynodioecious and subdioecious to nearly dioecious populations (Barrett, 1992). The geographical distributions of monomorphic and dimorphic populations of *W. dioica ssp. alba* overlap throughout the northern half of their range (see Fig. 1, and Barrett, 1992); however, gender dimorphism is associated with relatively drier soil conditions at both broad and local scales (Barrett, 1992; Case and Barrett, 2001). Phylogenetic evidence based on both morphological and molecular data suggests that populations of these sexual systems, although very closely related, may represent distinct taxa rather than populations of the

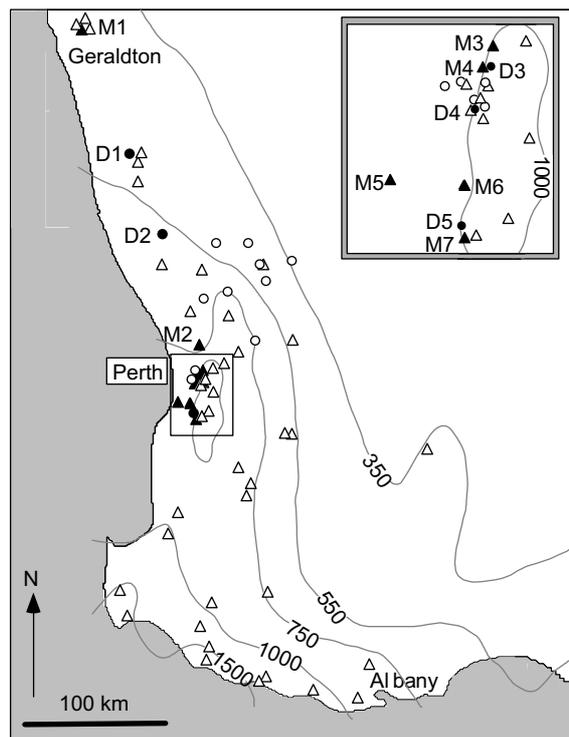


Figure 1. The distribution of monomorphic (triangles) and dimorphic (circles) sexual systems of *W. dioica ssp. alba* in southwestern Australia. We identified populations using information from published species descriptions and monographs (Macfarlane, 1980, 1987), from label information on specimens lodged at the West Australian Herbarium, from field notes of S.C.H. Barrett and T.D. Macfarlane (pers. comm.), and from field explorations in 1995 and 1996. Closed symbols indicate populations sampled for this study. Isohyets indicating mean annual rainfall (in mm) are redrawn from figure 1 in Foulds (1993).

same subspecies (Case *et al.*, unpub. Ms). However because our molecular sampling of populations of the two sexual systems is limited, as is our information about levels of gene flow or cross compatibility, we maintain the nomenclature of the most recent taxonomic treatment of this group (Macfarlane, 1980, 1987) for this study.

Here we assess patterns of gender expression and allocation among 12 monomorphic and dimorphic populations of *W. dioica* ssp. *alba* to determine whether the sexual systems exhibit distinct biomass allocation patterns, and whether these respond to latitudinal changes in water availability. We exploited a distinct rainfall gradient in southwestern Australia. Southern portions of the range of *W. dioica* ssp. *alba* experience considerably higher rainfall than in the more arid north. We were particularly interested in how life-history traits likely to contribute to relative female fitness, including allocation to female function, flower size and number, and the allocation of biomass to growth vs. reproduction, might differ among populations exposed to different amounts of rainfall. In our study, we addressed the following specific questions: (1) Do the gender morphs of *W. dioica* differ in overall allocation patterns and can they be distinguished based on size-related traits? Distinct allocation patterns that vary with gender, rather than population location, indicate differentiation between sexual systems with respect to resource use, as well as their relative tolerance to resource limitation. (2) How does gender expression vary along the precipitation gradient? We predicted that aridity should favor higher female frequencies and lower fruiting by males in dimorphic populations, but that cosexuals would maintain higher proportional investment in ovules. (3) How do biomass allocation patterns of the sexual systems respond to differences in precipitation? We predicted that with lower precipitation in the north, plants should favor below ground structures, to enhance resource acquisition, and lower total reproductive investment, with fewer or smaller flowers, in comparison with plants from populations farther south.

Methods

Study species and sampling

Wurmbea dioica plants are composed of an underground corm and an annual shoot, consisting of two or three linear to lanceolate leaves and a terminal cymose inflorescence with an average of 2–3 small white actinomorphic flowers, which can be pistillate, staminate, or perfect (Macfarlane, 1980). In monomorphic populations, most plants produce only perfect flowers; staminate flowers occasionally occur at distal positions on the inflorescence and on small individuals. In dimorphic populations, plants produce either all pistillate

(females), or varying proportions of staminate and perfect flowers (males). Hereafter, we follow the convention of Lloyd and Bawa (1984) in referring to all polleniferous plants in dimorphic populations as males, and all plants of monomorphic populations as cosexuals.

We sampled populations in 1995 and 1996 at peak flowering along a 450 km north–south transect between Geraldton and Perth, WA (closed symbols, Fig. 1). This sample spans the region of overlap in the distributions of the two sexual systems. Mean annual precipitation increases from ~350 mm in the south-central coast region to more than 1500 mm in the southwest corner (Foulds, 1993, see Fig. 1). Data from Automatic Weather Stations throughout the study region confirm this precipitation pattern for 1995 and 1996 (courtesy of Agriculture Western Australia, Case 2000).

Analysis of geographical variation

We compared morphological variation, biomass allocation, and gender expression of flowering plants among seven monomorphic and five dimorphic populations of *W. dioica* using both discrete and continuous size-related traits. In each population we recorded flower number, flower size, and the sexual condition of all flowers (pistillate, staminate, or perfect) on 30–52 plants of each gender. Whole plants bearing single flowering shoots were also collected (15–52 per gender morph per population), washed free of soil, and dried to constant weight. We divided each plant into its component parts: below ground (roots and corms), above ground vegetative (stems and leaves), and reproductive (flowers and associated inflorescence parts) and measured the length and width of parts using either digital calipers or a plastic ruler and then weighed them to the nearest 0.1 mg using an electronic balance.

We used multivariate techniques to assess the extent to which monomorphic and dimorphic populations of *W. dioica* can be distinguished based on variation in 16 morphological and life-history traits associated with contrasting biomass allocation patterns. Various size-related characters, including corm size and burial depth, length and width (both blade and swollen base) of each of the three leaves, leaf and flower spacing, and flower size, were scaled, centered, purged of multivariate outliers, and then subjected to a principle components analysis and a one-way MANOVA in JMP (Version 3.2.2, SAS Institute, 1997) to determine the extent to which the sexual systems differ in these attributes. We additionally performed a discriminant function analysis to predict sexual system and gender morph based on a linear combination of the traits measured.

We employed nested ANOVA and ANCOVA in JMP to analyze variation in biomass allocation and gender expression among sexual systems, populations within sexual systems, and gender morphs within populations (all fixed effects).

We used ANOVAs to test for differences among groups with respect to total biomass, and proportional allocation to below ground structures, above ground vegetative mass, and reproductive mass at flowering. All traits were either arcsine transformed (proportions) or natural log transformed (all other traits) prior to analysis to meet assumptions of normality. For analyses of all other traits, we included total biomass as a covariate; interactions between the covariate and the main effects were tested and eliminated from the model when not significant at $p < 0.05$ (Sokal and Rohlf, 1995).

For traits that varied significantly among populations in the overall ANOVAs, we used linear trend analysis (Kirk, 1995) to assess whether among-population differences within sexual systems were linearly associated with latitude. Trend analysis is a contrast, or multiple comparison (such as Tukey's test), which employs an F -test to compare three or more means that have known weights or coefficients. We used the CONTRAST statement in PROC GLM in SAS (Version 7.0, SAS Institute, 1999) and provided orthogonal polynomial coefficients for each population that were adjusted (following procedures in Kirk, 1995) to account for the uneven distribution of populations along the latitudinal gradient (Fig. 1). Note that because we used natural populations (as opposed to planting individuals at various latitudes), significant associations with latitude may be influenced by concomitant changes in abiotic factors (such as precipitation), as well as effects such as population age or evolutionary history if these also vary with latitude.

Results

Morphological differentiation

Monomorphic and dimorphic populations of *W. dioica* were highly differentiated with respect to vegetative and reproductive morphology (Fig. 2). Principle component analysis revealed that all 16 traits contributed to the first three principle components, which accounted for 66% of the variation in the dataset (Table 1). Ten traits related to above ground size largely comprised the first principle component, explaining more than 42% of the variation, while below ground size contributed heavily to both the second and third principle components. Above ground traits were significantly different between the sexual systems (MANOVA $F_{9,270} = 50.12$, $p < 0.0001$), and using discriminant functions analysis plants were reliably assigned to the appropriate sexual system (dimorphic: 96%, monomorphic: 92%, $n = 280$ plants) using the 10 traits related to above ground size (see Table 1). Repeating the discriminant functions analysis using three traits (leaf area, stem height, and flower size) highly

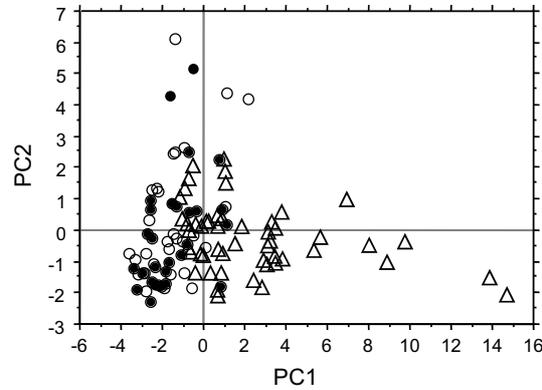


Figure 2. Scatterplot of plants in monomorphic and dimorphic populations of *W. dioica* ssp. *alba* subjected to principle components analysis of 16 morphological traits. Females (open circles), males (closed circles) and cosexuals (triangles) are shown with respect to the first two principle components. Eigenvectors are provided in Table 1.

Table 1. Principle component analysis of morphometric variation among seven monomorphic and five dimorphic populations of *Wurmbea dioica* ssp. *alba* in southwestern Australia

	PC 1	PC 2	PC 3
Eigenvalue	6.7416	2.4542	1.2924
% variation explained	42.1349	15.3389	8.0776
Cumulative % explained	42.1349	57.4738	65.5515
<i>Eigenvectors</i>			
Corm size	-0.10682	0.27277	0.35382
Corm depth	-0.03701	0.41495	-0.04744
Leaf length - 1	0.04883	0.57332	-0.17523
Leaf blade width - 1	0.26032	-0.00990	-0.09379
Leaf base width - 1	0.32084	-0.12341	-0.14500
Distance leaf 1 to 2	-0.03952	0.36181	-0.19977
Leaf length - 2	0.18743	0.46932	-0.08064
Leaf blade width - 2	0.31594	-0.03509	-0.19877
Leaf base width - 2	0.28290	-0.13316	-0.35480
Distance leaf 2 to 3	0.28232	0.07839	0.20494
Leaf length - 3	0.29418	0.12024	0.05222
Leaf blade width - 3	0.28493	0.05115	-0.07529
Leaf base width - 3	0.33582	-0.07489	-0.00152
Distance leaf 3 to fl 1	0.33030	-0.05138	0.18400
Flower size	0.32027	-0.02977	0.19716
Flower spacing	0.16034	0.08315	0.69073

Eigenvectors greater than 0.25 for the first three principle components (PC) are shown in **bold**.

correlated to these 10 yielded similar results, sexual system was predicted accurately 92% of the time.

Although the gender morphs also differed significantly for above ground traits (MANOVA $F_{9,115} = 6.38$, $p < 0.0001$), there was a greater degree of

morphological similarity between females and males than there was between unisexuales and cosexuals (Fig. 2). Assignment to a particular gender morph was less accurate than was the determination of sexual system (cosexual: 91%, female: 73%, male: 74% using all 10 above ground traits, cosexual: 88%, female: 54%, male: 59% using only leaf area, stem height and flower size). For both datasets, females and males were more likely to be misclassified to the other unisexual morph (23–41%) than determined to be cosexual (3–6%), attesting to the relative similarity of female and male morphologies.

In accord with these results, cosexuals had greater total leaf area (monomorphic = $33.7 \pm 1.01 \text{ cm}^2$, dimorphic = $23.6 \pm 1.68 \text{ cm}^2$, $F_{1,262} = 5.39$, $p = 0.021$), and were significantly taller (monomorphic = $7.48 \pm 0.14 \text{ cm}$, dimorphic = $4.58 \pm 0.22 \text{ cm}$, $F_{1,294} = 169$, $p < 0.0001$) than unisexuales, females and males did not differ with respect to leaf area ($F_{5,262} = 1.02$, $p = 0.41$), but males were taller than females in three of the five dimorphic populations (Pops. 3, 4, and 5, $F_{5,294} = 2.54$, $p = 0.028$). Inflorescences of plants of both sexual systems contained similar numbers of flowers ($F_{1,304} = 1.46$, $p = 0.23$). We detected a significant difference between the sexual systems in the effect of total plant mass on flower size (biomass * sexual system interaction: $F_{1,304} = 8.94$, $p = 0.003$) with the positive effect stronger among cosexuals (slope = 0.23 ± 0.02 , $R^2 = 0.36$) than among unisexuales (slope = 0.15 ± 0.02 , $R^2 = 0.24$). Although this interaction precludes testing for an overall difference in flower size between the sexual systems (while adjusting for variation in plant size), the fact that there was relatively little overlap in flower size measurements of plants from monomorphic vs. dimorphic populations indicates that cosexuals produce larger flowers than unisexuales.

Latitudinal variation in gender expression

We found striking differences in gender expression with latitude between the two sexual systems of *W. dioica*. As predicted, dimorphic populations farther north were characterized by a greater frequency of female plants ($r = -0.9386$, $p = 0.018$, Fig. 3A) and male plants with lower investment into female function (linear trend $F_{1,115} = 7.33$, $p = 0.008$, Fig. 3B). In contrast, cosexuals in all populations produced similar proportions of perfect flowers (linear trend $F_{1,190} = 0.55$, $p = 0.46$, Fig. 3C).

Latitudinal variation in biomass allocation

Total biomass differed significantly among populations, but not between the sexual systems or among the gender morphs (Table 2). Plants in southern populations of both sexual systems were larger in terms of total biomass than plants in more northern populations (linear trends, dimorphic: $F_{1,137} = 24.64$,

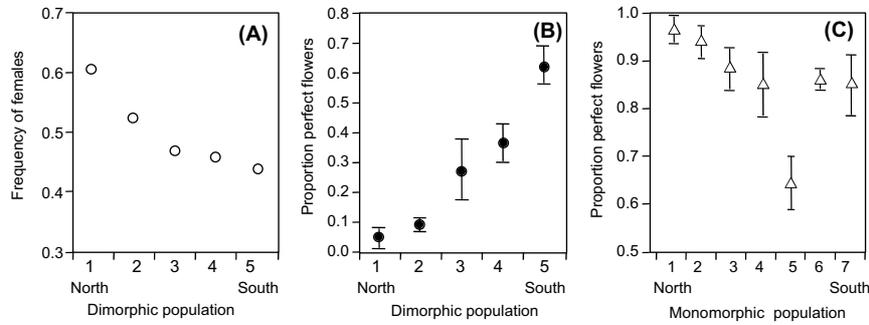


Figure 3. (A) Female frequency and (B) the proportion of perfect (hermaphrodite) flowers produced by males and (C) cosexuals in five dimorphic and seven monomorphic populations of *Wurmbea dioica* ssp. *alba* in southwestern Australia. Populations are ordered from north to south.

Table 2. ANOVA of biomass allocation patterns of monomorphic and dimorphic populations of *Wurmbea dioica* ssp. *alba* in southwestern Australia

Source	Total biomass	Below ground mass	Vegetative mass	Reproductive mass
Sexual system	0.36	269*	271*	85.6*
Population (sexual system)	20.2*	34.6*	42.0*	15.6*
Gender (pop, sexual system)	0.95	1.97	0.99	3.62**

We assessed variation between sexual systems ($df = 1$), among populations within sexual system ($df = 10$), and among genders within populations ($df = 5$) in total biomass, and proportions of biomass allocated below ground vs. above ground to vegetative and reproductive structures. Response variables were either natural log transformed (total biomass) or arcsine transformed (all others) to meet assumptions of normality. F values are shown, significance levels are indicated by superscripts: * $p < 0.0001$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.05$. All significant effects remain so after Bonferroni correction for multiple comparisons (Rice, 1989).

$p < 0.0001$, monomorphic: $F_{1,173} = 33.56$, $p < 0.0001$), although the positive trend was much steeper among dimorphic than monomorphic populations (Fig. 4A). Overall, the sexual systems exhibited distinct patterns of mean proportional allocation to each component of total mass (Table 1). Plants in dimorphic populations had larger corms, and allocated less biomass to above ground vegetative and reproductive mass at flowering than plants in monomorphic populations (Fig. 4B–D). Although females allocated more biomass to flowers than males (Table 1), this result was strongly influenced by a single dimorphic population (Pop. 3, Fig. 4D).

Patterns of biomass allocation varied from north to south but the sexual systems responded differently to the latitudinal gradient (Fig. 4B–D). Plants in monomorphic populations had proportionately more below ground biomass and allocated less to above ground vegetative and reproductive structures with

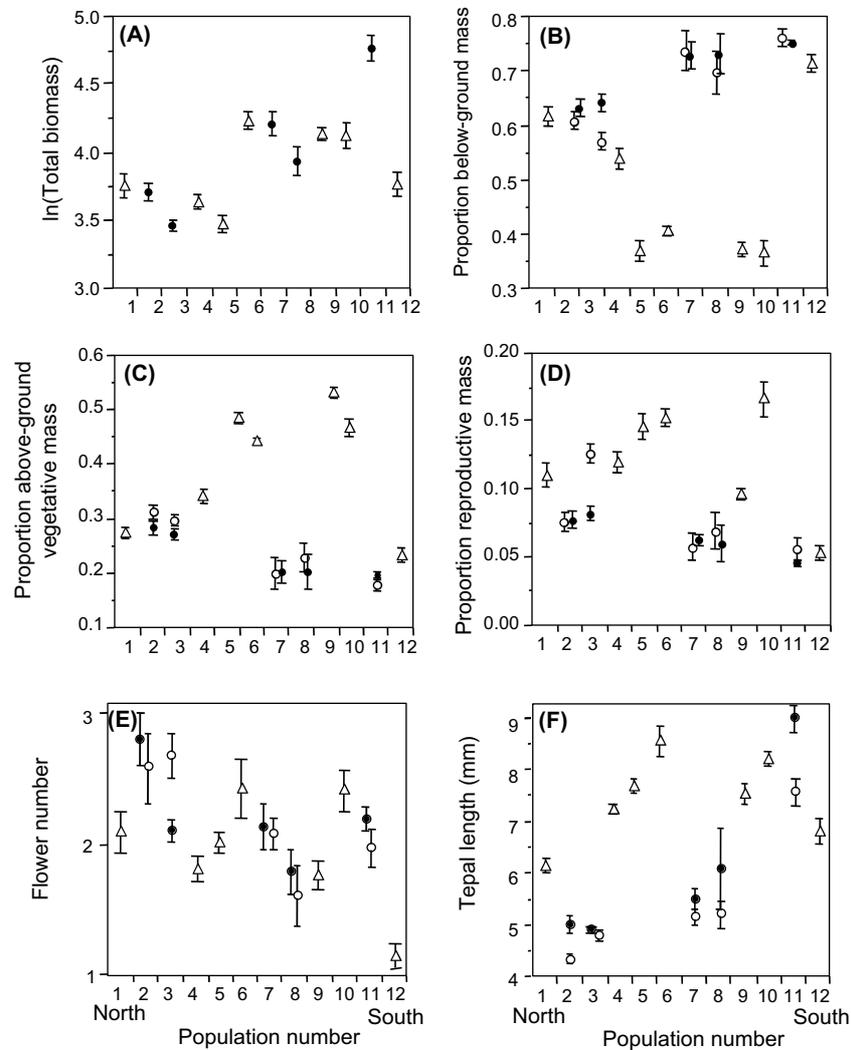


Figure 4. (A) Mean total biomass, (B) proportional allocation to below ground, (C) above ground vegetative and (D) reproductive mass, (E) flower number and (F) tepal length of plants in five dimorphic and seven monomorphic populations of *Wurmbea dioica* ssp. *alba* in southwestern Australia. Population means (and standard errors) are ordered from north to south. A. Plants in dimorphic are shown as open circles, plants in monomorphic populations are shown as triangles. B–F. Females are shown as open circles, males as closed circles, cosexuals as triangles.

decreasing latitude ($F_{1,173} = 58.66$, $p < 0.0001$, $F_{1,173} = 35.46$, $p < 0.0001$, and $F_{1,173} = 47.46$, $p < 0.0001$, respectively, Fig. 4B and C). In contrast, we found only two significant trends in mean proportional allocation among dimorphic populations. Females in more northern populations had less below ground mass ($F_{1,69} = 4.75$, $p = 0.033$, Fig. 4B) but greater reproductive mass

at flowering ($F_{1,69} = 13.63$, $p = 0.0004$, Fig. 4D, all other $p > 0.39$, Fig. 4) than populations in the southern portion of the range.

Flower number and flower size varied among monomorphic populations in the overall analysis ($F_{1,190} = 13.42$, $p = 0.0003$ and $F_{1,188} = 15.22$, $p < 0.0001$, respectively; Fig. 4E and F) with cosexuals in more southern populations producing larger flowers ($F_{1,188} = 31.58$, $p < 0.0001$) but not more flowers than plants in northern populations. Females but not males showed a marginally significant reduction in flower number with increasing latitude (females: $F_{1,92} = 3.31$, $p = 0.07$; males: $F_{1,115} = 1.82$, $p = 0.18$, Fig. 4E), while flower size in males increased significantly in southern populations (females: $F_{1,88} = 2.16$, $p = 0.14$; $F_{1,104} = 17.12$, $p < 0.0001$, Fig. 4F).

Discussion

The overall goal of this study was to assess how stress conditions might play a role in the evolutionary transition from cosexuality to dioecy via the gynodioecy pathway. Our approach involved investigating geographical variation in gender expression and allocation patterns of monomorphic and dimorphic sexual systems of *Wurmbea dioica* ssp. *alba* across a latitudinal precipitation gradient in southwestern Australia. Our studies revealed that the two sexual systems consistently exhibit contrasting allocation strategies. More importantly, the gender expression and sex allocation of males in dimorphic populations and cosexuals in monomorphic populations responded differently to stress conditions, despite the fact that both are phenotypically hermaphroditic. Earlier, we identified three mechanisms by which aridity could affect relative female fitness, easing conditions for the evolutionary transition between gender monomorphism and dimorphism: (1) resource limitation of female function in cosexuals; (2) gender-specific allocation strategies enhancing the growth and survival of females; (3) effects on pollination ecology, eliciting changes in the mating system. Below, we discuss the main findings of this study in relation to these hypotheses.

Resource limitation of female function

Allocation to female function in male and cosexual plants of *W. dioica* ssp. *alba* differed in response to the latitudinal gradient in precipitation (Fig. 3). Elsewhere, studies of several gynodioecious species have also reported a decline in the female fertility of males with increasing aridity and, correlatively, an increasing frequency of female plants in populations (Webb, 1979; Arroyo and Squeo, 1990; Delph, 1990b; Wolfe and Shmida, 1997; Ashman, 1999; Alonso and Herrera, 2001; Delph and Carroll, 2001; Asikainen and Mutikainen, 2003).

The fact that these patterns were also evident in *W. dioica* ssp. *alba* is significant because in all other species studied to date, males produce only perfect flowers and the regulation of female fertility results from variable levels of fruit set. In contrast, in *W. dioica* ssp. *alba*, variation in female fertility first occurs at the stage of ovary development, with males and cosexuals producing variable proportions of staminate and perfect flowers. This indicates that the effects of resource limitation on female function is not simply a reflection of variation in fruiting success and can be manifested much earlier in floral development.

Only males of *W. dioica* ssp. *alba* in dimorphic populations produced fewer perfect flowers at lower latitudes (Fig. 3B). In contrast, cosexuals produced consistently high proportions of perfect flowers across the study region (Fig. 3C), despite reductions in both precipitation and total plant size. This result is in accord with earlier findings by Delph (1990a, b) in New Zealand *Hebe* (Scrophulariaceae). She found that fruit production by males in gynodioecious *H. strictissima* and *H. subalpina* was strongly affected by stem size, and showed a higher degree of reproductive plasticity than that of conspecific females, or of cosexuals in closely related monomorphic species. She concluded that plasticity in fruit set must have developed after the evolution of gynodioecy, and therefore could not be invoked to explain the initial invasion of females into monomorphic populations (summarized in Delph, 2003). Our results support this conclusion for *W. dioica* ssp. *alba*. In contrast, Costich (1995) found evidence for size-dependent gender modification and resource limitation of fruit production in cosexual plants of *Ecballium elaterium* (Cucurbitaceae). Unlike *Wurmbea* and *Hebe*, cosexual *E. elaterium* plants have unisexual rather than perfect flowers. The gender expression of monoecious plants is strongly influenced by environmental conditions and species commonly display size-dependent gender modification (Webb, 1999; Sarkissian *et al.*, 2001; Dorken and Barrett, 2003).

Our estimates of investment in female function involved the proportion of perfect flowers per inflorescence and we found a latitudinal response among males but not cosexuals. It is possible that the female fitness of both polleniferous morphs varied with latitude, if the effect in cosexuals were manifested through reduced ovules per flower, or lower fruit or seed production in drier sites. However, our previous work indicates that where males and cosexuals occur sympatrically, they do not differ significantly in percent fruit set or seed production per fruit, despite the fact that cosexuals produce a greater proportion of perfect flowers and more ovules per flower than males (Case and Barrett, 2001 and 2004). Furthermore, Vaughton and Ramsey (2002) found that ovuliferous flower production and ovules per flower showed similar patterns along a latitudinal gradient among *W. biglandulosa* populations in New South Wales. This may indicate that the proportion of perfect flowers is a reasonable estimator of female fecundity.

There are two likely explanations for the differential effect of resource limitation on males and cosexuals. First, the gender morphs probably respond differently to resource limitation because of the mode through which each gains the majority of their reproductive fitness (Lloyd, 1974, 1976, 1980). On average, cosexuals gain fitness equally through female and male function. At the population level, we would expect to see reductions in allocation to both pollen and ovules such that proportional investment in sex function does not change under stress conditions. In contrast, males gain the majority of their fitness through pollen because of the large supply of ovules available on female plants. Males may be expected to favor reductions in ovule production rather than pollen production when resources become limiting (e.g., Delph, 1990b, c). Second, the reduction in female function among males in dimorphic populations may result from higher female frequencies with decreasing latitude, alone or in combination with the effects of resource limitation in the environment. The presence of females in dimorphic populations increases the pollen fitness of males as a result of frequency-dependent selection, and may favor genotypes with higher pollen production. Given a resource trade-off between female and male function, this effect would result in reduced seed production by males as females become more abundant (Lloyd, 1976; Charlesworth and Charlesworth, 1978; Maurice *et al.*, 1993; Ashman, 1999).

Gender-specific allocation strategies

Plants in monomorphic and dimorphic populations of *W. dioica* ssp. *alba* were differentiated with respect to above ground vegetative and reproductive traits. Plants could be reliably assigned to the appropriate sexual system based on stem height, leaf area, and flower size alone (Fig. 2). Patterns of biomass allocation in *W. dioica* ssp. *alba* indicate differentiation between the sexual systems both in overall allocation patterns and in their response to latitudinal changes in precipitation. Although all plants were larger in more southern (wetter) populations, plants in monomorphic populations favored allocation above ground, producing larger leaves, taller stems, and larger flowers than plants in dimorphic populations across their range (Fig. 4). These results are identical to allocation patterns we observed for the two sexual systems of *W. dioica* ssp. *alba* when they are found growing in sympatry (Case and Barrett, 2001). Unisexual plants occupy drier microsites and exhibit physiological and allocation patterns that favor resource acquisition and storage at the expense of investment into leaves and flowers. In contrast, cosexual plants occur in wetter microsites, and maintain high above ground allocation to taller stems, and to larger leaves and flowers. These results support the view that these contrasting patterns of biomass allocation in *W. dioica* ssp. *alba* reflect gender-specific strategies for coping with resource limitation and environmental heterogeneity. Common garden

studies would be required to determine how much of the observed phenotypic variation among populations has a genetic basis.

Reduced leaf area and a high root:shoot ratio are common responses of plants to limiting water availability (Schultze *et al.*, 1987). High below ground mass is likely to enhance resource uptake and storage, and should therefore be beneficial under lower resource conditions. Our prediction that plants should increase their below ground mass in drier areas was only confirmed in cosexual plants (Fig. 4B). In fact, unisexual plants showed the opposite response, maintaining greater below ground mass in all populations but particularly in those farther south. An allocation strategy favoring high investment below ground may be an inherent feature of gender dimorphism in *W. dioica* ssp. *alba* under a range of environmental conditions, and may afford unisexuals an advantage in terms of growth and survival in harsh habitats. However, it remains unclear why unisexuals might increase their resource storage under wetter conditions. More extensive sampling of populations would be useful to confirm this pattern.

High below ground allocation in dimorphic populations of *W. dioica* ssp. *alba* and the advantages it provides under harsh conditions may simply be a consequence of unisexuality. Females and males in all populations maintained greater below ground mass relative to cosexuals (Fig. 4B), which likely reflects the ability of unisexuals to acquire and store resources from the soil. Although this allocation strategy may provide an advantage to unisexual plants throughout their range, proportional allocation strategies between the sexual systems were most similar in the northernmost (driest) populations we studied. Males differed from cosexuals in their response to changes in precipitation with respect to biomass allocation. Males exhibited plasticity in allocation to female function, but not in overall biomass allocation patterns, while the opposite was true for cosexuals. It is unlikely that biomass allocation patterns alone reflect growth and survival, as we found a higher frequency of female plants where below ground mass was smallest (i.e., northernmost populations). Thus, it would be worth investigating gender-based differences in physiology along this gradient, as dynamic processes, e.g., rates of photosynthesis or resource use efficiency, are likely to contribute a great deal to the overall efficiency of growth processes.

Influence of stress on pollination and mating

In insect-pollinated plants, phenotypic reductions in flower size as a result of environmental stress have the potential to promote greater levels of inbreeding, with implications for the evolution of gender dimorphism. Smaller flower size increases the likelihood of intra-floral selfing because of the reduced spatial separation between the sex organs (Webb and Lloyd, 1986), or by making smaller-bodied insects more effective pollen vectors. Although selfing and

inbreeding depression are generally considered a prerequisite for the evolution of dioecy from cosexuality (reviewed in Charlesworth, 1999), the proximate ecological mechanisms that promote increased selfing rates are still poorly understood. Stress-related changes in floral traits of cosexual *W. dioica* ssp. *alba* may alter mating patterns to the extent that it provides the necessary stimulus for the selection of unisexuality.

We found no evidence for an effect of aridity on flower number (Fig. 4E), but lower precipitation was associated with significant reductions in flower size in both cosexual and male plants (Fig. 4F). Under some ecological circumstances, such as dry habitats, smaller flower size may be beneficial because it can reduce water loss through flowers (Galen, 2000). In *W. dioica* this phenotypic change may also be associated with mating costs, particularly inbreeding. *Wurmbea dioica* flowers have nectaries on each tepal that are situated beneath the anther sacs, and reduced flower size brings the nectaries into closer proximity to dehiscent anthers. Small nectar-foraging flies, the primary pollinators of dioecious populations, are abundant throughout southwestern Australia and forage unsystematically for long periods within individual plants (Case and Barrett, 2004). Flies foraging for nectar in the larger flowers of cosexual plants, where bees are the predominant pollinators, are usually too small to be effective pollinators. However, stress-induced reductions in flower size seem likely to promote increased rates of self-pollination by rendering flies more effective pollen vectors. A change in pollination and mating patterns in small-flowered cosexual populations could provide an outbreeding advantage to female plants, favoring the establishment of gender dimorphism. According to this hypothesis, interactions between stress conditions, floral traits, and pollinators could play a role in the evolution and maintenance of alternative sexual systems in *W. dioica* ssp. *alba*.

Comparisons and future studies

This investigation represents one of only three studies to date assessing the response of related sexually monomorphic and dimorphic plant populations to variation in stress conditions. Costich (1995) studied ecological differentiation of monoecious and dioecious subspecies of *Ecballium elaterium* in the Iberian Peninsula in relation to aridity. In common with our study, she found no differences in the size of plants in monomorphic vs. dimorphic populations, and that plants of both sexual systems were larger at wetter sites. However, there are several important differences between the findings of these two studies. In *E. elaterium*, drier conditions were associated with decreased flower number for all gender morphs (but particularly males), and with reduced allocation to female function in cosexual plants. In contrast, in *W. dioica* drier conditions were associated with reduced flower size not flower number for all genders, and we

found no effect of aridity on the production of ovuliferous flowers by cosexuals. These differences probably result from the strikingly different life histories of the two species (*E. elaterium* is a large prostrate herb rather than a diminutive geophyte as in *W. dioica*) and contrasts in the reproductive performance of hermaphroditic plants with perfect vs. unisexual flowers.

Vaughton and Ramsey (2002) report among-population gender variation ranging from cosexuality to gynodioecy in *Wurmbea biglandulosa* ssp. *biglandulosa* in eastern Australia. Gynodioecious populations of this species occur in areas with significantly lower precipitation than monomorphic populations. However, there was no evidence for an effect of precipitation on population sex ratio, or covariation between female frequency and male seed production, as reported here and for several other gynodioecious species (Delph, 1990a; Wolfe and Shmida, 1997; Ashman, 1999; Alonso and Herrera, 2001; Delph and Carroll, 2001; Asikainen and Mutikainen, 2003). These contrasting results may be explained by the different patterns of gender variation that occur within populations of *W. dioica* and *W. biglandulosa*. With one exception, dimorphic *W. biglandulosa* populations contain few staminate-flowered individuals; nearly all polleniferous plants bear perfect flowers. This indicates that *W. biglandulosa* is closer to the 'gynodioecious end' of the gender spectrum between gynodioecy and dioecy (see Lloyd, 1976). In contrast, the patterns of gender expression in *W. dioica* from Western Australia indicate that populations function closer to subdioecy. If males in *W. biglandulosa* gain more of their total fitness via seed than males of *W. dioica*, then allocation to ovules by *W. biglandulosa* males may be maintained despite the imposition of ecological stress.

The aridity gradient we investigated correlates with gender variation among dimorphic populations of *W. dioica* ssp. *alba* (Fig. 3) and provides a plausible ecological mechanism for how gender specialization might evolve. However, our results provide little support for the hypothesis that environmental stress has contributed directly to the origin of gender dimorphism in this species through resource limitation of female allocation in cosexual populations. Instead our results raise the intriguing possibility of more complex interactions involving the influence of stress on floral traits resulting in altered pollination and mating patterns. Future studies examining interactions between abiotic and biotic factors are likely to provide novel insights into the ecological mechanisms responsible for the evolution of gender dimorphism.

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