

## ECOLOGICAL DIFFERENTIATION OF COMBINED AND SEPARATE SEXES OF *WURMBEA DIOICA* (COLCHICACEAE) IN SYMPATRY

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**Abstract.** The evolution and maintenance of combined vs. separate sexes in flowering plants is influenced by both ecological and genetic factors; variation in resources, particularly moisture availability, is thought to play a role in selection for gender dimorphism in some groups. We investigated the density, distribution, biomass allocation, and physiology of sympatric monomorphic (cosexual) and dimorphic (female and male) populations of *Wurmbea dioica* in relation to soil moisture on the Darling Escarpment in southwestern Australia. Populations with monomorphic vs. dimorphic sexual systems segregated into wet vs. dry microsites, respectively, and biomass allocation patterns and physiological traits reflected differences in water availability, despite similarities in total ramet biomass between the sexual systems. Unisexuales flowered earlier at lower density, and they allocated significantly more biomass below ground to roots and corms than did cosexuals, which allocated more biomass above ground to leaves, stems, and flowers. Females, males, and cosexuals produced similar numbers of flowers per ramet, but unisexuales produced more ramets than cosexuals, increasing the total number of flowers per genet. Contrary to expectation, cosexuals had significantly higher (more positive) leaf carbon isotope ratios and lower leaf nitrogen content than unisexuales, suggesting that cosexuals are more water-use efficient and have lower rates of photosynthesis per unit leaf mass despite their occurrence in wetter microsites. Cosexuals appear to adjust their stomatal behavior to minimize water loss through transpiration while maintaining high investment in leaves and reproductive structures. Unisexuales apparently maximize the acquisition and storage of both water and nitrogen through increased allocation to roots and corms and enhance the uptake of CO<sub>2</sub> by keeping stomata more open. These findings indicate that the two sexual systems have different morphological and physiological features associated with local-scale variation in water availability.

**Key words:** carbon isotope ratios; cosexuality; geophyte; habitat specialization; leaf nitrogen content; plant gender; resource allocation; sexual systems; sympatry; unisexuality; water limitation; *Wurmbea dioica*.

### INTRODUCTION

The evolution of separate sexes from combined sexes has occurred many times among angiosperm families. The ecological and genetic conditions favoring these contrasting sexual systems therefore represent a central question in evolutionary biology (reviewed in Geber et al. 1999). Theoretical models have invoked three key factors affecting selection for gender dimorphism, including the genetic control of sex expression, the fitness consequences of selfing and outcrossing, and the optimal allocation of resources to female and male function (Charnov 1982, Lloyd 1982, Charlesworth 1999). Because ecological context can affect the relative importance of these factors, recent work on gender dimorphism has focused on the environmental conditions that contribute to the relative success of combined vs. separate sexes.

Numerous empirical studies have implicated ecological conditions, particularly harsh environments, in the evolution and maintenance of gender dimorphism (Webb 1979, Hart 1985, Arroyo and Squeo 1990, Delph 1990a, b, Barrett 1992, Costich 1995, Weller et al. 1995, Wolfe and Shmida 1997, Ashman 1999, Sakai and Weller 1999). These studies have reported increased gender specialization and a greater incidence of gender dimorphism in resource-limited habitats. For example, all of the dimorphic species of *Schiedea* (Caryophyllaceae) in Hawaii are found in dry habitats, whereas all but four monomorphic species occur in wet habitats (Weller and Sakai 1990). In the Iberian peninsula, the sexual systems of *Ecballium elaterium* (Cucurbitaceae) are geographically segregated, with the monoecious subspecies restricted to the wetter northern region and the dioecious subspecies occurring in the drier south (Costich 1995). In several dimorphic (gynodioecious and subdioecious) species, increased gender specialization (e.g., higher frequencies of female plants and reduced fruit production by hermaphrodites) is associated with more stressful environments (Webb 1979, Arroyo and Squeo 1990, Delph 1990a, Barrett 1992, Wolfe and Shmida 1997, Ashman 1999, Case 2000).

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Why should separate sexes be favored under harsh ecological conditions? Darwin (1877) was first to note that under resource-limited conditions, unisexuals would be favored over those investing in both pollen and seed if unisexuals could outperform hermaphrodites in the comparable sex function (i.e., reproductive compensation). This prediction has been supported by a recent theoretical model, which further proposes that the sex with the lower total reproductive expenditure should be favored in poor environments (De Laguérie et al. 1993). Delph (1990b) showed that the seed fitness of female plants relative to hermaphrodites is greatest in harsh environments in *Hebe strictissima* (Scrophulariaceae), and proposed that the mechanism for this effect involves greater plasticity of fruit set on hermaphrodites than females. The negative relation between reproductive investment and stress tolerance is often observed in dioecious species, where males, which do not bear the high cost of fruiting, are better able to tolerate low resource conditions than females (reviewed in Delph 1999). Within populations, male plants often occur in greater abundance in dry microsites (Freeman et al. 1976, Bierzychudek and Eckhart 1988, Dawson and Bliss 1989, Dawson and Ehleringer 1993), and many have sex-specific morphological and physiological mechanisms for dealing with drought (reviewed in Dawson and Geber 1999). Thus, gender-based differences in morphology and physiology, when associated with spatial segregation by habitat quality, may reflect the direct influence of disparate environmental conditions, or selection for strategies that maximize resource acquisition, improve resource use efficiency, or enhance survival under harsh environmental conditions.

These arguments based on studies of dioecious species may be extended to ecological studies of combined vs. separate sexes. Stressful habitats may contribute to the maintenance of gender dimorphism if: (a) limited resources reduce reproductive allocation, providing an advantage to gender specialists with lower total reproductive expenditure; or (b) poor microsite quality reduces the reproductive fitness of hermaphrodites relative to unisexuals (Delph 1990b). Thus, in comparing the ecology of monomorphic and dimorphic sexual systems among related species, we might expect gender dimorphism to occur more frequently in resource-limited conditions. Likewise, we may predict that plants occurring in resource-limited habitats would exhibit morphological and physiological traits improving resource acquisition and use, since total resource acquisition is both influenced by the availability of resources in the environment, and by the efficiency with which plants obtain those resources.

Because taxa with combined vs. separate sexes usually differ in a variety of life-history features, several studies have investigated intraspecific variability in sexual systems. Species with monomorphic and dimorphic sexual systems provide powerful models for

investigating the ecology of gender variation, because the transition between the two sexual systems has likely occurred relatively recently, and plants with combined vs. separate sexes should be similar in most other aspects of their morphology and ecology. However, relatively few such taxa are known (e.g., *Ecballium elaterium*, Costich 1995; *Elatostema* spp., Lahav-Ginot and Cronk 1993; *Leptinella* spp., formerly *Cotula*, [Asteraceae], Lloyd 1972; *Mercurialis annua* [Euphorbiaceae], Pannell 1996; *Sagittaria latifolia* [Alismataceae], Wooten 1971, Sarkissian et al. 2001; *Wurmbea dioica* [Colchicaceae], Barrett 1992), and rarely do the contrasting sexual systems occur sympatrically. Evidence for ecological differentiation and habitat specialization of sympatric monomorphic and dimorphic sexual systems would suggest an important role for resource availability in the evolution and maintenance of gender variation.

Here we investigate the ecology of monomorphic and dimorphic populations of *Wurmbea dioica* [(R. Br.) F. Muell] ssp. *alba* (Macfarlane) at a site where the sexual systems occur in sympatry. *Wurmbea dioica* ssp. *alba* is a diminutive, self-compatible, insect-pollinated geophyte that is widespread in southwestern Australia. Population surveys indicate continuous variation in sexual systems, ranging from monomorphic populations through gynodioecious and subdioecious to fully dioecious populations (Barrett 1992), with contrasting sexual systems occurring sympatrically at several sites near Perth, Western Australia (WA). Recent molecular studies of a monomorphic and a dimorphic population of *W. dioica* ssp. *alba* in WA indicate that the sexual systems differ by several mutations in a relatively conserved region of the chloroplast (Case 2000). This raises the possibility that they may represent different species. However, because our sampling to date is limited, we will follow recent taxonomic treatments (Macfarlane 1980, 1987) and refer to plants of the different sexual systems as *W. dioica* ssp. *alba*.

In the present study, we asked two specific questions. First, what is the pattern of density, distribution, and flowering phenology of monomorphic and dimorphic sexual systems in sympatry, and are these patterns related to variation in soil moisture or depth? Barrett (1992) reported ecological differentiation of the sexual systems of *W. dioica* ssp. *alba* in one sympatric site on the Darling Escarpment near Perth, WA. Plants in the dimorphic population occurred on shallow, rocky, well-drained soils and flowered earlier at lower density, whereas monomorphic populations inhabited waterlogged soils, flowered later, and occurred at higher density. Here, we quantified the patterns of ecological differentiation observed by Barrett (1992), including differences in flowering time, spatial distribution, population density, and soil moisture availability. Temporal segregation of the sexual systems in sympatry would reduce gene flow between them, likely contributing to the maintenance of ecological differentiation of the

sexual systems despite their close proximity. Spatial segregation associated with measurable differences in microsite quality implicates differential responses of the sexual systems to varying resource conditions, and possibly selection for contrasting adaptive strategies (Bierzychudek and Eckhart 1988, Dawson and Geber 1999).

Second, do plants in monomorphic and dimorphic populations differ in patterns of biomass allocation and physiology? Plants with greater reproductive expenditure should be under stronger selection to increase resource acquisition by: (a) specializing on high resource (i.e., wetter) microsites, and (b) favoring morphological and physiological strategies maximizing resource acquisition and storage (e.g., increased allocation to corms and roots, greater photosynthetic capacity) or more efficient resource use (e.g., greater water use efficiency). Morphological and physiological differences between the sexual systems may reflect either plastic responses to variation in resource levels among microsites, or gender-specific strategies for dealing with resource limitation. We assessed variation in morphology and physiology by measuring leaf nitrogen content as an estimate of photosynthetic capacity (Field and Mooney 1986, Evans 1989, but see Laporte and Delph 1996), and carbon isotope ratios ( $\delta^{13}\text{C}$ ) as an estimate of time-integrated water use efficiency (WUE; Farquhar et al. 1989), and relating these traits to biomass allocation patterns and variation in soil moisture.

## METHODS

### *Study species*

*Wurmbea dioica* plants are composed of an underground corm and an annual shoot, consisting of one basal plus two cauline linear leaves and an erect cymose inflorescence (Macfarlane 1980). Plants flower only in winter-wet areas of temperate Australia, and their corms consist of water (~45% by mass) and starch, but negligible mineral resources (Pate and Dixon 1982). Populations in Western Australia flower from June to early September depending on winter rainfall. Inflorescences contain a mean of 2–3 flowers (range 1–9) which can be pistillate, staminate, or hermaphroditic (perfect). 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; see Fig. 2 in Barrett 1992). 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. Fruits of both sexual systems mature in late September and October, with globose seeds shaken from the dry dehiscent capsules by wind. Plants of both sexual systems can undergo limited clonal growth, each episode

resulting in the production of one additional physiologically-independent ramet that does not disperse, but remains enclosed within the tunics covering the parent corm.

### *Study site*

We conducted our studies during the winters of 1995 and 1996 at a single site in a recreational area near Lesmurdie, WA, ~40 km east of Perth (32°00'27" S, 116°01'42" E). Populations of each sexual system at this site consisted of 8000–10 000 plants over ~2 ha; the frequency of female plants in the dimorphic population was 0.40. The site is characterized by open scrub of native vegetation (*Xanthorrhoea*, *Eucalyptus*, *Acacia*, and *Melaleuca* spp.) growing in rocky clay-loam over granite on the southwest face of the Darling Escarpment. Winter temperatures at this site range from about 8–17°C, with soil temperatures between 11–15°C. Total precipitation in the months of June, July and August was 175, 300, and 95 mm, respectively, in 1995, and 300, 238, and 159 mm in 1996 (data from Lesmurdie Automatic Weather Station, courtesy of Agriculture Western Australia, South Perth, Western Australia).

A large fire occurred at the site in the summer of 1994–1995, which removed much of the aboveground vegetation and stimulated the germination of serotinous seeds and the flowering of many geophytes (A. L. Case, *personal observation*). The removal of adult foliage from Australian communities increases water availability to plants growing after fire, an effect which declines as shrubby vegetation regenerates and increases evapotranspiration (Gill 1981, Wellington 1984, Hodgkinson 1992). Further, plants resprouting from perennating structures that maintain substantial root systems, such as corms or basal buds, have been shown to take better advantage of the increased water availability after fire than do seedlings with more limited root production (Jianmin and Sinclair 1993). Therefore, comparisons between the 1995 and 1996 seasons should reflect greater water availability to *W. dioica* plants in 1995 relative to 1996 when the shrubby vegetation had returned.

### *Density, distribution, and flowering phenology*

Plant density, distribution, and phenology were recorded using linear transects. In 1995, we established two permanent 40-m transects through areas of transition between monomorphic and dimorphic populations to assess density and distribution. We recorded the total number of plants present in each of 80 0.25-m<sup>2</sup> quadrats along each transect (total  $n = 160$  quadrats). The ends of the transects were permanently marked with steel rebars, and mapped using a GPS and compass directions to ensure relocation the following season. In 1996, we established eight additional 20-m transects located throughout the site (total  $n = 480$  quadrats). Transects were surveyed weekly to establish

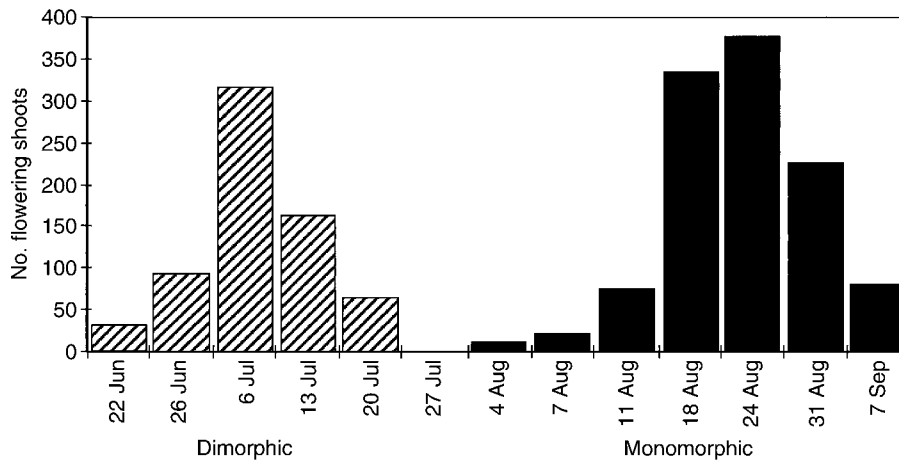


FIG. 1. Flowering phenology of sympatric dimorphic (hatched bars) and monomorphic (solid bars) populations of *Wurmbea dioica* at Lesmurdie, Western Australia, in 1996. The number of plants in each 0.25-m<sup>2</sup> quadrat was recorded weekly along 10 transects and summed to produce flowering curves for each population.

population-level flowering phenology as well as density and distribution in 1996.

We plotted flowering curves and transect data as frequency distributions to visually assess differences between monomorphic and dimorphic populations in flowering time and spatial distribution. We statistically compared measures of density both within and between seasons using a two-way analysis of variance in JMP (SAS Institute, 1997) with year (1995 vs. 1996), sexual system (monomorphic vs. dimorphic), and their interaction as fixed main effects.

#### Soil moisture availability and depth

We assessed variation in soil moisture among microsites containing plants of each sexual system in the 1996 season. We randomly placed 48 0.25-m<sup>2</sup> quadrats throughout the sympatric site. We recorded the number of plants in each quadrat and collected soil samples by inserting a 50-mL Falcon tube (Fisher Scientific, Pittsburgh, Pennsylvania) 4.1 cm into the soil (up to the 35-mL mark), resulting in a constant sample volume of 29 mL (mean [ $\pm 1$  SE] corm depth at this site was 3.61 [ $\pm 0.10$ ] cm,  $n = 111$  plants). All plots were  $\geq 10$  m apart over a total area of  $\sim 2$  ha, and all samples were collected on the same day between 1500 and 1800. Because of the difference in flowering time between monomorphic and dimorphic populations (see *Results*, Fig. 1), quadrats were sampled first on 2 July 1996 (during peak dimorphic flowering,  $n = 12$  plots), then again on 27 August 1996 (during peak monomorphic flowering,  $n = 18$  monomorphic and 18 dimorphic plots). Soil samples were taken within 2 cm of a *W. dioica* plant, tightly sealed with plastic wrap and placed in a sealed plastic container, taken immediately to the University of Western Australia Botany Department and weighed to the nearest 0.1 mg. Soil samples were dried at 50°C for 10 d in a drying oven, weighed, re-

turned to the oven for an additional 3 d, then reweighed to ensure the samples were completely dry. Soil water content was calculated gravimetrically ([wet mass – dry mass]/dry mass).

We measured soil depth beneath each quadrat for all transects and soil moisture plots by manually inserting a 20 cm long metal probe as far as possible into the soil. Depth measurements were taken at three locations within each quadrat, estimated to the nearest mm, and the mean was calculated. Each measurement was taken within 2 cm of a *W. dioica* plant, two beside plants nearest to opposite corners, and one nearest the center. This procedure better estimates the soil depth experienced by plants, and eliminates bias resulting from exposed rocks where plants could not grow. Sexual-system differences in both soil moisture content and soil depth of sampled quadrats were compared using *F* tests.

#### Biomass allocation patterns

We compared biomass allocation patterns of females, males, and cosexuals during flowering using both discrete and continuous size-related traits. In both 1995 and 1996, we recorded flower number, the sexual condition of each flower (pistillate, staminate, or perfect), and the number of ramets flowering per genet for 24–58 plants of each sex. In 1996, a similar-sized sample of plants of each sex was collected for analysis of biomass allocation patterns based on tissue dry masses. Whole plants of cosexuals, males, and females bearing single flowering ramets were collected during flowering, washed free of soil, and dried to constant mass. For each genet, we counted the number of ramets per genet, and classified each ramet as dormant, vegetative (producing leaves only), or reproductive. Each reproductive ramet (the parent ramet in all cases) was then divided into its component parts: belowground (roots and corms), aboveground vegetative (stems and

leaves), and reproductive (flowers and associated inflorescence parts). Roots were counted; corms, stems, leaves, flowers, and inflorescences were measured for length and width using either digital calipers or a plastic ruler, then weighed to the nearest 0.1 mg. Total leaf area per plant was estimated by summing leaf area estimates over each of the three linear leaves (area per leaf = total leaf length  $\times$  leaf width at base).

Variation in flower number, floral sex ratio, and the number of ramets per genet was analyzed using a nested ANOVA with year (1995 vs. 1996), sexual system (monomorphic vs. dimorphic), and gender within sexual system (female vs. male) as main effects. For biomass allocation patterns in 1996, we used ANCOVAs to compare the two sexual systems (monomorphic vs. dimorphic), and males and females in dimorphic populations, while adjusting for total biomass. 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). Adjusting for total mass permits comparison of allocation patterns among plants of different sizes, as well as enabling the evaluation of variation in allocation with changes in total plant size (i.e., biomass by main effect interactions).

Additionally, we assessed potential trade-offs between resource acquisition and reproductive allocation using reproductive allometries. We determined the slopes of the relation between the log of total reproductive mass at flowering and log of total nonreproductive mass. This eliminates potentially confounding effects of autocorrelation between flowering mass and total plant biomass (Samson and Werk 1986). Slopes of 1 indicate proportional increases in allocation to flowers with increasing size, while slopes greater than one indicate that floral allocation increases disproportionately with size. Allometries with slopes less than one suggest trade-offs between size and reproductive allocation during flowering.

All response variables and covariates were natural-log-transformed to meet assumptions of normality. The number of roots and flowers per ramet were not normally distributed even after transformation, however, results from analyses of untransformed data matched those of natural-log-transformed and square-root-transformed data. In all analyses, males were considered a single gender category, regardless of whether they produced any perfect flowers. This classification is justified because polleniferous individuals of *W. dioica* in eastern Australia have been shown to alter their sex expression between reproductive episodes, such that males that produce seed in one season may produce only pollen the next, and vice versa (Barrett et al. 1999). Furthermore, in this population males that produced seed did not differ significantly from those that produced only pollen in any aspect of size (contra Barrett et al. 1999), and inclusion of the additional sex class ("fruiting males") did not affect conclusions from any of the analyses.

#### *Carbon isotope ratio and leaf nitrogen content*

To compare physiological traits between monomorphic and dimorphic sexual systems in 1996, one to five whole plants were collected from each of the 36 0.25-m<sup>2</sup> plots (18 monomorphic and 18 dimorphic) from which soil samples were taken in August (see *Methods: Soil moisture availability and depth*). Because plants from monomorphic and dimorphic plots were at different developmental stages during August, an additional 14 male and 14 female plants were collected during peak dimorphic flowering in July for comparison at the flowering stage. These plants were analyzed for biomass allocation patterns, leaf nitrogen content, and carbon isotope ratio ( $\delta^{13}\text{C}$ ). The ratio of  $\text{C}^{13}$  to  $\text{C}^{12}$  in the structural components of leaves serves as an indicator of relative stomatal aperture, and thus reflects long term WUE of the leaf (Farquhar et al. 1989). More positive  $\delta^{13}\text{C}$  values indicate greater stomatal closure, i.e., greater WUE.

The dried basal leaf of each plant was sent to the Environmental Isotopes Laboratory at the University of Waterloo (Waterloo, Ontario, Canada) for determination of  $\delta^{13}\text{C}$  by mass spectrometry (Isochrom Continuous Flow Stable Isotope Mass Spectrometer [Micromass UK, Manchester, UK] coupled to Carlo Erba Elemental Analyzer CHNS-O EA1108 using Carlo Erba Elemental Standards B2005, B2035, and B2036 [CE Instruments, Milan, Italy]). Stomatal behavior, hence  $\delta^{13}\text{C}$ , is influenced by climatic conditions, particularly humidity and temperature, and can vary among leaves of different positions (Farquhar et al. 1989). This variation should be minimized for the August sampling date because plants of both sexual systems were collected at the same time and site, and because we used leaves at equivalent developmental positions on each plant.

We used leaf nitrogen content per unit dry mass to estimate photosynthetic capacity (Field and Mooney 1986, Evans 1989). The lower cauline leaf (the second leaf) of each plant was kept in a drying oven overnight at 45°C, weighed to the nearest 0.01 mg (sample masses ranged from 0.4 to 5.6 mg), then digested using a modified micro-Kjeldhal technique. Leaf samples were placed in 14-cm test tubes, to which 10 mg zinc dust, and 450 mL concentrated sulfuric acid were added. After 5 min, the samples were capped with glass drip bulbs and heated at the highest setting for 20 min in an aluminum heat block on a hot plate. We allowed the samples to cool 5 min before adding 100 mg potassium sulfate to the mixture. Samples were heated at 250°C until clear, swirling to mix every 20 min. Samples took 2–3 h to clear, depending on the size of the leaf tissue. Each sample was diluted to a total volume of 10 mL with double distilled H<sub>2</sub>O; total leaf N was measured as total NH<sub>4</sub> per sample using an Technicon AAII Auto Analyser (Technicon Instrument, Saskatoon, Saskatchewan, Canada).

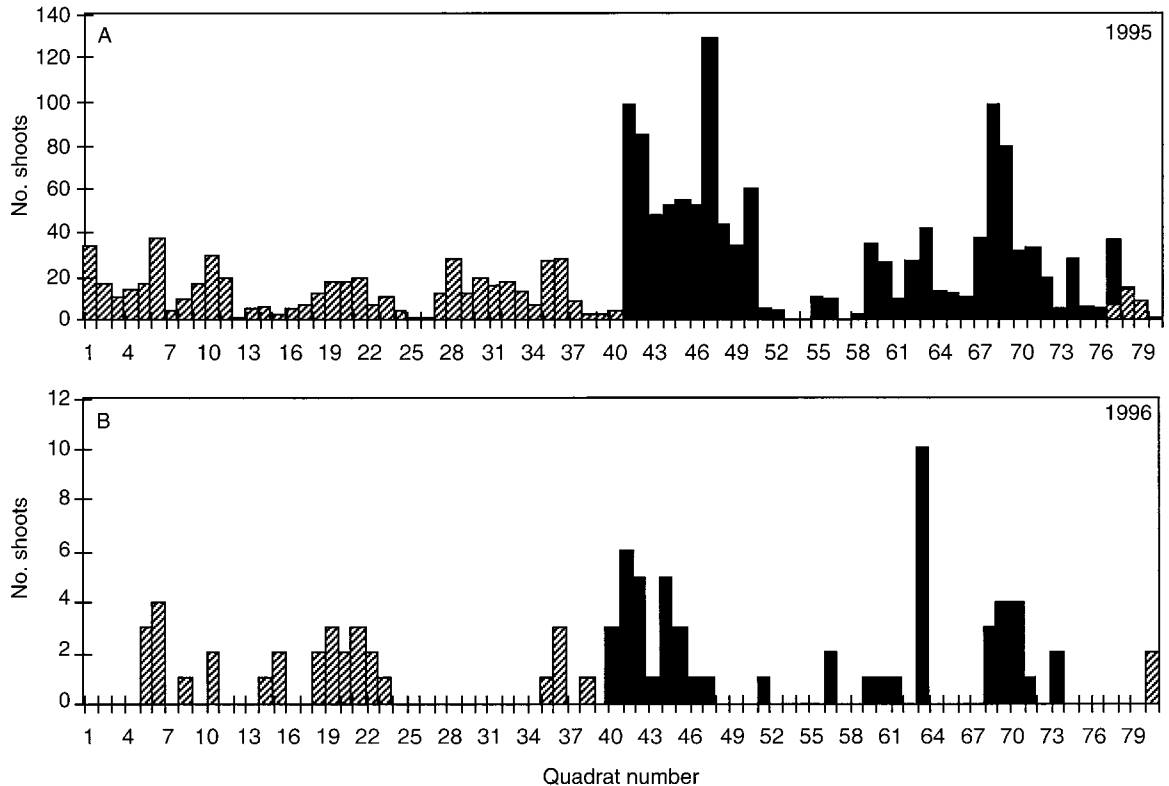


FIG. 2. Density and distribution of sympatric dimorphic (hatched bars) and monomorphic (solid bars) populations of *Wurmbea dioica* at Lesmurdie, Western Australia, in (A) 1995 and (B) 1996. The number of plants in each 0.25-m<sup>2</sup> quadrat was recorded along 40-m transects through areas of transition between monomorphic and dimorphic populations. Data shown are from one representative transect sampled in both years.

Because  $\delta^{13}\text{C}$  and leaf N content were measured on two sampling dates for females and males and only once for cosexuals, these data were analyzed using two separate ANOVAs. A two-way ANOVA comparing the physiology of plants in the dimorphic population between sampling dates included gender (female vs. male), sampling date (July vs. August), and their interaction as fixed main effects. To test for differences between the sexual systems in August, we used a nested ANCOVA, with sexual system (monomorphic vs. dimorphic) and gender within sexual system (female vs. male) as main effects, and soil moisture content as a covariate. We used correlation analysis to assess potential relations between leaf N,  $\delta^{13}\text{C}$ , and allocation patterns at flowering. Corm size, root number, above-ground vegetative mass, reproductive mass, leaf N, and  $\delta^{13}\text{C}$  were regressed on total biomass to remove the effects of total size, and the residuals from these relations were used to calculate Pearson correlations for each pair of traits; all residuals were significantly normally distributed using a Shapiro-Wilk test in JMP. The significance of the correlations were adjusted for multiple comparisons using sequential Bonferroni correction (Rice 1989).

## RESULTS

### *Density, distribution, and flowering phenology*

Dimorphic and monomorphic populations in sympatry showed no overlap in flowering time, and a high degree of spatial segregation (Figs. 1 and 2). In 1996, plants in the dimorphic population flowered from mid-June until late July, and finished flowering before cosexuals began in early August (Fig. 1). However, the duration of the flowering period was similar for both sexual systems, about one month. In both 1995 and 1996, unisexuals were widespread throughout the site at low density, while cosexuals were localized in a few, relatively small (<20 m in diameter), high-density patches. The density and distribution pattern of the sexual systems is evident from transect data, an example of which is shown in Fig. 2, and which is representative of the 10 transects sampled. In 1995, only 13.8% of quadrats contained plants of both sexual systems, and none contained plants of both sexual systems in 1996, indicating minimal overlap in distribution. We did not quantify other aspects of distribution within the site, but did note that cosexuals were seldom found on

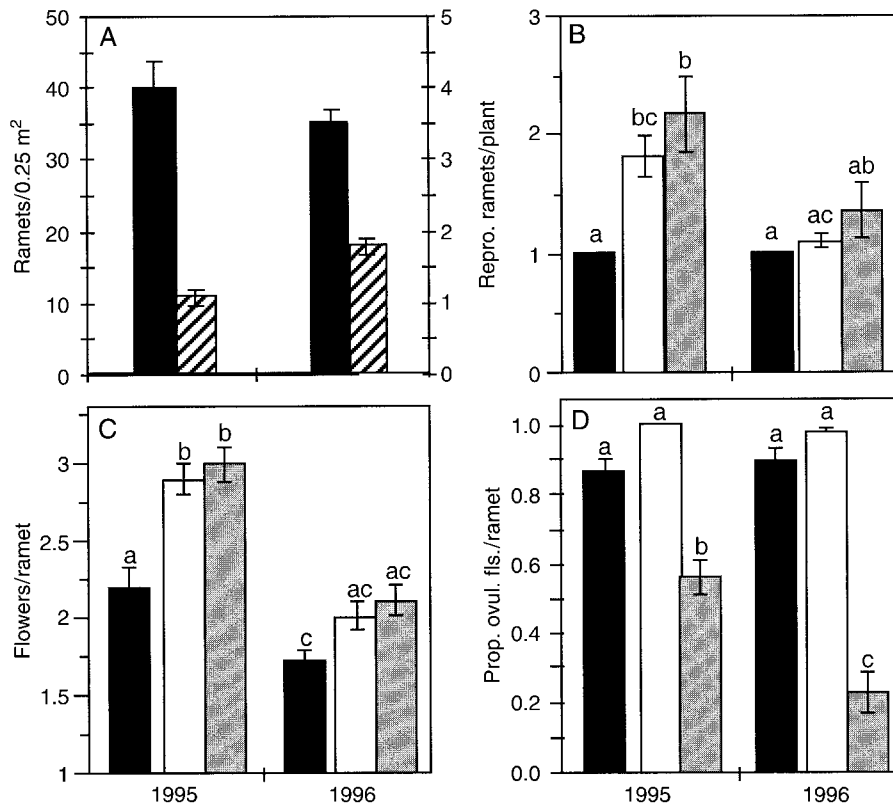


FIG. 3. Reproductive traits of sympatric monomorphic and dimorphic populations of *Wurmbea dioica* at Lesmurdie, Western Australia, in two consecutive years following fire. (A) Means ( $\pm 1$  SE) for the number of ramets flowering/0.25 m<sup>2</sup> are shown for monomorphic (solid bars) and dimorphic (hatched bars) populations; note the difference in scale for 1995 (left abscissa) and 1996 (right abscissa). (B) Number of flowering ramets per plant (means  $\pm 1$  SE) are shown. (C) Number of flowers per ramet (means  $\pm 1$  SE) is shown. (D) Floral sex ratios (means  $\pm 1$  SE) are shown for cosexuals (black bars), females (white bars), and males (gray bars). All means  $\pm 1$  SE. Letters among genders for each trait in both years indicate means that were significantly different from one another in Tukey-Kramer tests.

steep slopes, where females and males were found in abundance.

Between years and between sexual systems, we detected significant differences in density and the number of reproductive ramets per genet (Fig. 3). In both years, cosexuals occurred at 2–4 times higher density than unisexuals; there was a ten-fold decrease in the density of cosexual ramets and a five-fold decrease in the density of unisexual ramets per quadrat from 1995 to 1996 (year:  $F_{1,176} = 36.9$ ,  $P < 0.0001$ ; sexual system:  $F_{1,176} = 14.3$ ,  $P < 0.001$ ; year  $\times$  sexual system:  $F_{1,176} = 12.3$ ,  $P < 0.001$ ; Fig. 3A). Because cosexual genets produced only one flowering ramet per season, the decrease in the density of the monomorphic population resulted from fewer genets flowering in 1996 (Fig. 3B). In contrast, we observed both females and males with up to 16 reproductive ramets in each year (sexual system:  $F_{1,261} = 11.9$ ,  $P < 0.0006$ ; year  $\times$  sexual system:  $F_{1,261} = 4.05$ ,  $P < 0.05$ ), and on average, unisexual genets had more simultaneously flowering ramets in 1995 than in 1996, which likely contributed to the difference in the density of the dimorphic population between years (Fig. 3B).

Plants of all genders produced more flowers in 1995 than 1996 ( $F_{1,261} = 48.3$ ,  $P < 0.0001$ ; Fig. 3C). In both years, cosexuals and females produced a high proportion of ovuliferous flowers (perfect or pistillate, respectively; Fig. 3D). Male plants produced fewer ovuliferous flowers than the other gender morphs in both years (sexual system:  $F_{1,259} = 34.1$ ,  $P < 0.0001$ ; gender within sexual system:  $F_{1,259} = 200$ ,  $P < 0.0001$ ) and produced fewer in 1996 compared with males flowering in 1995 (year  $\times$  gender:  $F_{1,259} = 14.0$ ,  $P < 0.001$ ; Fig. 3D).

#### Soil water availability and depth

The sites where unisexuals occurred were characterized by significantly drier, shallower soils than microsites where cosexuals occurred. The microsites of unisexuals were significantly drier than those of cosexuals when both were measured in August ( $F_{1,45} = 10.4$ ,  $P = 0.0023$ ), although within the dimorphic population, microsites were significantly wetter in August than in early July ( $F_{1,45} = 39.3$ ,  $P < 0.0001$ ; Fig. 4). The difference in water content that was observed between monomorphic and dimorphic populations in Au-

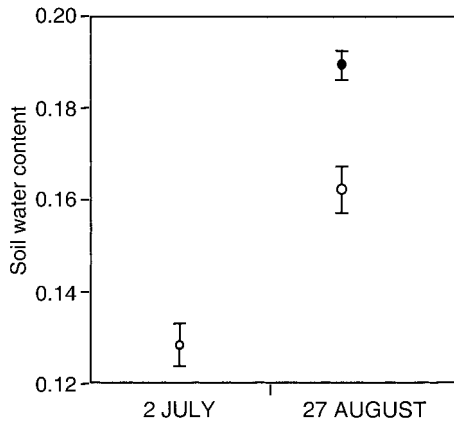


FIG. 4. Soil water content of microsites occupied by monomorphic (closed circle) and dimorphic (open circles) populations of *Wurmbea dioica* in sympatry on the Darling Escarpment, Western Australia. Soil moisture content was measured gravimetrically:  $(\text{wet mass} - \text{dry mass})/\text{dry mass}$ . See *Methods* for details of sampling. Means for soil water content ( $\pm 1$  SE) are shown. Monomorphic populations could not be sampled on 2 July because they had not emerged above the soil.

gust amounts to  $\sim 1$  mL of water per 29 mL soil. Plants in the dimorphic population were found in significantly shallower soils than plants in the monomorphic population (mean depth  $\pm$  SE for dimorphic,  $9.45 \pm 0.22$  cm; monomorphic,  $11.25 \pm 0.25$  cm;  $P < 0.001$ ).

#### Biomass allocation

In 1996, flowering ramets from monomorphic and dimorphic populations were not significantly different with respect to total biomass ( $F_{1,75} = 0.05$ ,  $P > 0.90$ ), but all three genders differed significantly in biomass allocation patterns (Figs. 5 and 6). As total size increased, unisexual ramets had larger corms (biomass  $\times$  sexual system:  $F_{1,75} = 28.6$ ,  $P < 0.0001$ ; Fig. 5A) and allocated less biomass to aboveground vegetative (biomass  $\times$  sexual system:  $F_{1,72} = 26.4$ ,  $P < 0.0001$ ; Fig. 5B) and reproductive mass at flowering (biomass  $\times$  sexual system:  $F_{1,72} = 18.4$ ,  $P < 0.0001$ ; Fig. 5C)

than cosexual ramets. Additionally, male ramets increased aboveground vegetative and flowering mass with increasing size, whereas female ramets did not (biomass  $\times$  gender:  $F_{1,72} = 6.9$ ,  $P < 0.01$  and  $F_{1,72} = 5.7$ ,  $P < 0.05$ , respectively; Fig. 5B and C). Slopes of reproductive allometries (i.e., log reproductive mass vs. log nonreproductive mass) closely matched the patterns shown in Fig. 5C. Among reproductive ramets, males and cosexuals exhibited significant positive slopes ( $m = 1.52 \pm 0.09$ ,  $n = 50$ ,  $P < 0.0001$  and  $m = 0.98 \pm 0.28$ ,  $n = 14$ ,  $P = 0.0049$  respectively), while the slope among females was not significantly different from zero ( $m = 0.21 \pm 0.31$ ,  $n = 14$ ,  $P > 0.50$ ).

All measured traits (see Table 1) were significantly positively related to total biomass, but size relations differed between the sexual systems for all traits except root number and flower size (Table 1). Among plants having only one reproductive shoot in 1996, the total number of ramets was 1–2 among cosexuals and 1–4 among unisexuals. Clonality was significantly positively related to the total mass of the parent ramet among cosexuals ( $m = 0.30 \pm 0.088$ ,  $R^2 = 0.20$ ,  $P = 0.0013$ ), and more so among males ( $m = 1.84 \pm 0.47$ ,  $R^2 = 0.56$ ,  $P = 0.0021$ ), but not among females ( $m = 0.62 \pm 0.64$ ,  $R^2 = 0.07$ ,  $P > 0.30$ ).

Accounting for the effect of size, flowering cosexual ramets had significantly fewer roots and larger flowers than unisexual ramets, while females and males did not differ significantly for either trait (Table 1; root number: cosexual =  $8.06 \pm 0.32$ , female =  $10.07 \pm 0.58$ , male =  $9.00 \pm 0.58$ ; tepal length: cosexual =  $8.17$  mm  $\pm 1.02$ , female =  $5.21$  mm  $\pm 1.03$ , male =  $5.42$  mm  $\pm 1.03$ ). Stem height and leaf area increased significantly with size among cosexuals, but not among unisexuals, and cosexuals were significantly taller with more leaf area than unisexuals (Fig. 6A and B). Both cosexuals ( $m = 2.07 \pm 0.18$ ,  $R^2 = 0.74$ ,  $P < 0.0001$ ) and males ( $m = 1.14 \pm 0.41$ ,  $R^2 = 0.39$ ,  $P < 0.017$ ) increased flower number per ramet with increasing size, while females did not ( $m = 0.015 \pm 0.27$ ,  $R^2 = 0.0003$ ,  $P > 0.95$ ; Fig. 6C). Flower mass increased with size

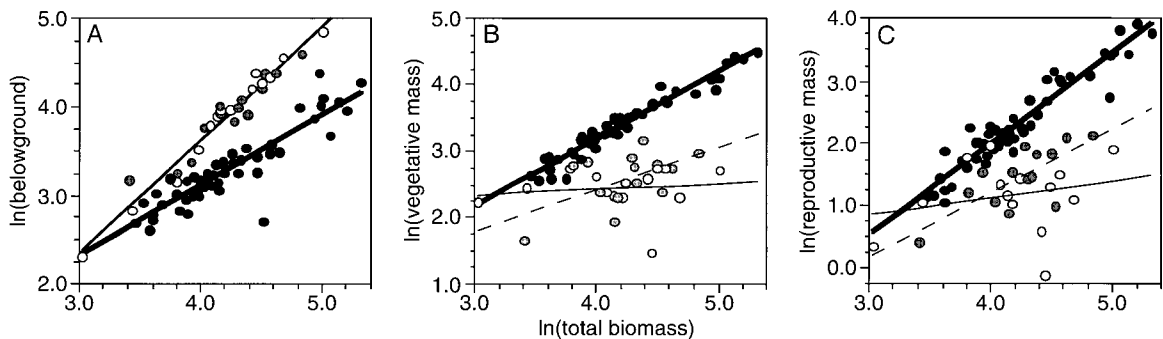


FIG. 5. Size dependence of biomass allocation to (A) belowground, (B) aboveground vegetative, and (C) reproductive structures at flowering for sympatric monomorphic and dimorphic populations of *Wurmbea dioica* near Perth, Western Australia, in 1996. Slopes for female (white circles, thin solid lines), male (gray circles, dashed lines), and cosexual ramets (black circles, thick solid lines) are shown where significantly different.



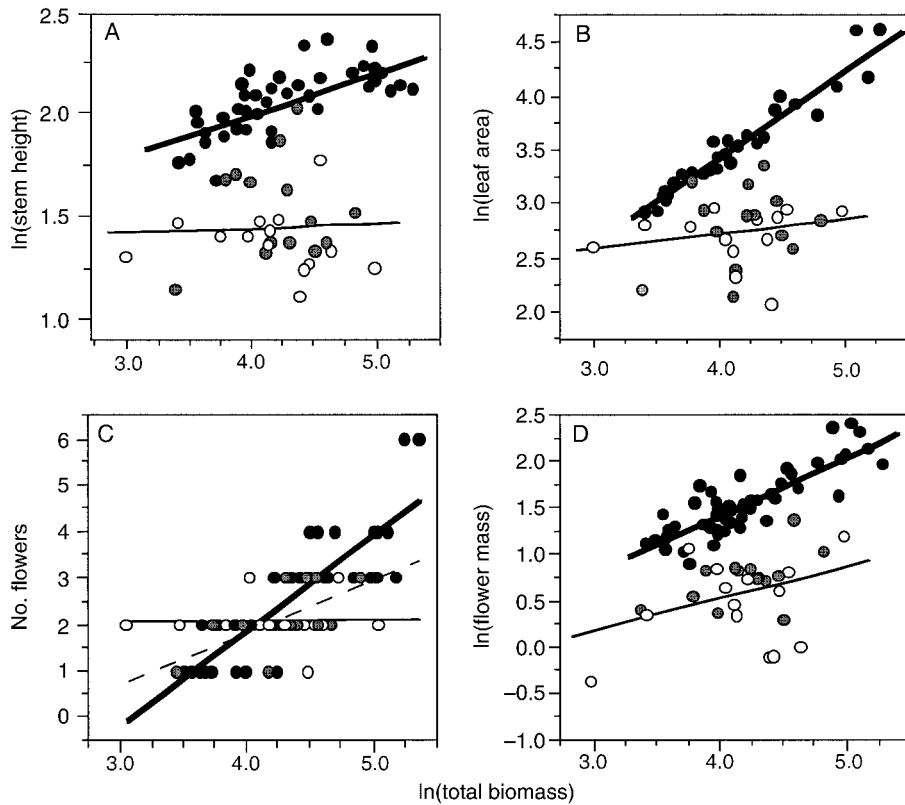


FIG. 6. Size-related traits of sympatric monomorphic and dimorphic populations of *Wurmbea dioica* at Lesmurdie, Western Australia. Relations with total biomass are shown for (A) stem height, (B) leaf area, (C) flower number, and (D) flower mass. Slopes for female (white circles, thin solid lines), male (gray circles, dashed lines), and cosexual ramets (black circles, thick solid lines) are shown where significantly different.

for both sexual systems, although more for cosexuals ( $m = 0.62 \pm 0.056$ ,  $R^2 = 0.72$ ,  $P < 0.0001$ ) than for unisexuals ( $m = 0.35 \pm 0.17$ ,  $R^2 = 0.14$ ,  $P < 0.048$ ; Fig. 6D).

#### Carbon isotope ratio and leaf nitrogen content

Carbon isotope ratios ( $\delta^{13}\text{C}$ ) varied significantly between sampling dates and between the sexual systems (Table 2). Females and males had more positive  $\delta^{13}\text{C}$  in July (at flowering) than in August (i.e., their stomata

were more open during fruiting;  $F_{1,43} = 29.8$ ,  $P < 0.0001$ ). In August, cosexuals (at flowering) had significantly higher  $\delta^{13}\text{C}$  than unisexuals ( $F_{1,38} = 5.4$ ,  $P < 0.03$ ), although in pairwise comparisons females were not significantly different from either males or cosexuals (Table 2).

Within the dimorphic population, leaf nitrogen (N) content did not differ between females and males at either sampling date ( $F_{1,43} = 1.9$ ,  $P > 0.17$ ), or between unisexuals in flowering and fruiting phase ( $F_{1,43} = 0.51$ ,

TABLE 1. ANOVA of size-related traits of sympatric monomorphic and dimorphic populations of *Wurmbea dioica* near Perth, Western Australia.

Trait	Sexual system	Gender	Biomass	Biomass $\times$ sexual system	Biomass $\times$ gender
Ramets per genet	5.31*	5.09*	21.7****	7.97**	4.88*
Root number	7.94**	1.67	4.8*	...	...
Stem height	0.62	7.91**	7.26**	6.54*	...
Leaf area	13.9****	0.79	49.9****	27.2****	...
Flowers per ramet	19.8****	4.25*	69.4****	22.1****	4.38*
Flower mass	0.23	6.68*	45.8****	4.25*	...
Tepal length	221****	0.92	43.0****	...	...

Notes:  $F$  values are shown. We assessed variation between sexual systems ( $df = 1$ ), among genders within sexual systems ( $df = 1$ ), and the effect of total biomass ( $df = 1$ ) on several size-related traits.

\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , \*\*\*\*  $P < 0.0001$ .

TABLE 2. Leaf characteristics related to physiological efficiency of sympatric females, males, and cosexuals of *Wurmbea dioica* in Lesmurdie, Western Australia.

Trait	Female	Male	Cosexual
July			
$\delta^{13}\text{C}$	-27.48* (0.22)	-27.00* (0.22)	
Foliar N (mg N/g leaf tissue)	63.89 (3.49)	57.46 (2.52)	
Leaf specific mass (mg/cm <sup>2</sup> )	1.84 (0.09)	2.09 (0.13)	
August			
$\delta^{13}\text{C}$	-28.44* <sup>ab</sup> (0.21)	-28.67* <sup>a</sup> (0.25)	-27.78 <sup>b</sup> (0.21)
Foliar N (mg N/g leaf tissue)	60.15 <sup>a</sup> (4.10)	55.41 <sup>a</sup> (6.12)	34.72 <sup>b</sup> (0.78)
Leaf specific mass (mg/cm <sup>2</sup> )	1.71 <sup>ab</sup> (0.19)	2.08 <sup>a</sup> (0.16)	1.48 <sup>b</sup> (0.08)

Notes: Means (with 1 SE in parentheses) for leaf-specific mass, carbon isotope ratio ( $\delta^{13}\text{C}$ ), and foliar nitrogen content are shown for females and males during flowering (July) and early fruiting (August), and for cosexuals during flowering (August). Asterisks within columns indicate significant differences between sampling times within gender morphs at  $P < 0.01$ ; superscript letters within rows indicate significant differences among gender morphs at  $P < 0.01$ .

$P > 0.45$ ). Between the sexual systems, the leaves of females and males contained ~70% more N per g of leaf tissue than the leaves of cosexuals ( $F_{1,38} = 31.6$ ,  $P < 0.0001$ ; Table 2), and cosexuals had lower specific leaf masses (i.e., their leaves were thinner) than unisexuals ( $F_{1,69} = 12.9$ ,  $P < 0.0006$ ; Table 2).

Significant correlations among physiological and allocation traits were few, and those that were significant differed between the sexual systems (Table 3). Leaf N content varied significantly with allocation only among females, where plants with greater vegetative mass had lower leaf N, while  $\delta^{13}\text{C}$  was not correlated with any other variable in any sex. After controlling for the effects of total biomass, larger corm size was associated with reduced vegetative and reproductive mass in both females and males, implying relatively strong trade-offs between belowground and aboveground components of resource investment. In all three sexes, vegetative and reproductive mass were significantly positively correlated, suggesting that increased vegetative mass, likely resulting from greater leaf area, increases resource availability for reproduction.

#### DISCUSSION

Monomorphic and dimorphic sexual systems of *Wurmbea dioica* differ ecologically in sympatry. Gender dimorphism was associated with early flowering, lower plant density, drier microsites, lower water use efficiency and higher leaf nitrogen content (thus, probably higher rates of photosynthesis), and allocation patterns that favor belowground resource acquisition and storage at the expense of investment into leaves and flowers. In contrast, gender monomorphism was associated with higher plant density, wetter microsites, greater investment into leaves and flowers, and physiological mechanisms maximizing water use efficiency. Below we contrast morphological and physiological features of monomorphic and dimorphic sexual systems of *W. dioica*, and discuss their implications for the maintenance of combined vs. separate sexes.

#### Ecological differentiation of the sexual systems

Our results demonstrate striking spatial and temporal segregation of the sexual systems of *W. dioica* in sympatry. Unisexuals flowered in late June and early July, and were widespread at low density throughout the drier areas of the study site, while smaller high-density patches of cosexuals flowered in late July and early August in wetter microsites, hence there was minimal opportunity for reproductive interactions between plants of the two sexual systems. These patterns are consistent with our observations at four other sites along the Darling Escarpment where the sexual systems occur in sympatry (Barrett 1992; A. L. Case and S. C. H. Barrett, *unpublished data*), suggesting that ecological segregation is a general feature of these sympatric populations.

Segregation of flowering time in sympatry (Fig. 1) likely maintains ecological and genetic differentiation between the sexual systems of *W. dioica* by restricting opportunities for hybridization. In allopatric populations elsewhere in Western Australia (WA), this phenological pattern does not occur; both monomorphic and dimorphic sexual systems in a given geographical region flower at the same time (A. L. Case and S. C. H. Barrett, *unpublished data*). In the Perth region during August, allopatric populations of both sexual systems flower concurrently with sympatric monomorphic populations. This suggests that the flowering time of dimorphic populations in sympatry shifted earlier in the season, and not that monomorphic populations flower later, or that both populations have altered phenological schedules. Earlier flowering may be related to the drier soil conditions experienced by dimorphic populations compared with monomorphic populations in sympatric sites. Elsewhere there is considerable evidence for an association between early flowering and drought stress in herbaceous plants (reviewed in Rathcke and Lacey 1985, Guerrant 1989, Diggle 1992). Allopatric populations of both sexual systems of *W. dioi-*

TABLE 3. Pearson pairwise correlations between time-integrated water use efficiency ( $\delta^{13}\text{C}$ ) and total leaf nitrogen content (mg N/g leaf tissue), root number, and components of biomass (mg) of sympatric females, males, and cosexuals of *Wurmbea dioica* in Lesmurdie, Western Australia.

A) Females (below diagonal) and males (above diagonal), measured in July						
Traits	Leaf N	$\delta^{13}\text{C}$	Corm mass	Root number	Vegetative mass	Reproductive mass
Leaf N	...	0.01	0.58	-0.41	-0.57	-0.40
$\delta^{13}\text{C}$	0.07	...	-0.31	-0.14	0.18	0.18
Corm mass	0.37	-0.15	...	-0.07	-0.96*	-0.73*
Root number	-0.08	0.13	0.55	...	0.11	-0.10
Vegetative mass	-0.78*	0.004	-0.76*	-0.11	...	0.75
Reproductive mass	-0.48	0.02	-0.70**	-0.28	0.81	...

B) Cosexuals, measured in August					
Traits	$\delta^{13}\text{C}$	Corm mass	Root number	Vegetative mass	Reproductive mass
Leaf N	0.36	0.002	-0.33	0.10	0.14
$\delta^{13}\text{C}$	...	-0.01	-0.36	-0.06	0.38
Corm mass	...	...	0.40	0.41	0.21
Root number	...	...	...	-0.15	-0.37
Vegetative mass	...	...	...	...	0.67*

Note: Correlations are shown for females and males at flowering (July;  $n = 14$ ) and cosexuals at flowering (August;  $n = 18-20$ ).

\*  $P < 0.05$ ; \*\*  $P < 0.10$ , following sequential Bonferroni correction.

*ca* flower earlier in the drier, northern parts of its range in Western Australia than populations in wetter southern regions (A. L. Case and S. C. H. Barrett, *unpublished data*). Yet, differences in soil moisture alone are unlikely to explain the complete lack of overlap between the sexual systems in sympatry. Asynchronous flowering among sympatric species is often caused by selection against the inferior products of hybridization (reviewed in Grant 1971, Arnold 1997), and this could also be the case for *W. dioica*, especially if plants of the two sexual systems turn out to be from separate biological species.

Spatial segregation of combined and separate sexes into wet vs. dry habitats, such as we found in sympatric populations of *W. dioica* (Figs. 2 and 4), also occurs in monoecious and dioecious subspecies of *Ecballium elaterium* (Costich 1995). However, segregation in *E. elaterium* occurs at a much larger geographical scale, and is likely maintained by differential requirements for seed germination or differences in dispersal ability (Costich 1995). Spatial segregation on a local scale has often been reported for females and males of dioecious species, where males are frequently found in drier and females in wetter microsites (reviewed in Bierzychudek and Eckhart 1988, Iglesias and Bell 1989, Dawson and Geber 1999). This pattern is often attributed to the higher reproductive cost of females, which may increase mortality in resource-limited sites, or to differential effects of aridity on female and male fitness (but see Iglesias and Bell 1989). Microsite effects on seed germination, seedling establishment, or mortality are possible mechanisms maintaining segregation in sympatric populations of *W. dioica*. However, without manipulative experiments, it is difficult to determine

whether the gender morphs suffer differential mortality in different microsites, have competitive advantages over one another, or experience changes in fitness among sites (Bierzychudek and Eckhart 1988, Weller and Sakai 1990). Because plants of *W. dioica* are difficult to grow in culture and transplants do not thrive under field conditions (A. L. Case and S. C. H. Barrett, *unpublished data*; T. D. Macfarlane, *personal communication*), we have no data addressing this issue.

As we predicted, differences in plant density between the sexual systems were associated with soil moisture, the dimorphic population occurring at lower density in drier sites than the monomorphic population. Low resource availability may limit the number of flowering individuals that can be supported in a particular habitat, through effects on seed germination, competition, or mortality (Harper 1977, Krischik and Denno 1990), or because resource limitation alters flowering schedules, such that only a fraction of individuals reproduce in any given season (Meagher 1984). It seems unlikely that flowering schedules are responsible for the difference in flowering density between sexual systems, as the pattern was evident following fire in 1995 when a large fraction of the total population was likely stimulated to flower. Moreover, differences in female fertility cannot account for the higher density of cosexuals because female ramets produce, on average, four times as many seeds as cosexuals (Case 2000). Microsite effects on seed germination and seedling establishment could account for the patterns of density as well as the spatial segregation of sexual systems.

Regardless of the ecological mechanisms responsible for differences in flowering phenology and plant density, these patterns may contribute to the maintenance

of monomorphic and dimorphic populations by influencing pollination and mating. Variation in resource availability at a local scale may control selection for gender variation both directly, through effects on resource allocation (see Delph 1990b), and indirectly, through effects on pollination. Shifts in pollinator service from "specialist" to more "generalist" pollen vectors in stressful habitats have been associated with transitions to dimorphic sexual systems in several plant species (Ganders 1978, Delph 1990b, Weller and Sakai 1990, reviewed in Sakai and Weller 1999). Although the sexual systems of *W. dioica* occur in sympatry, they do not share the same pollinator pools because of differences in flowering time. While early-flowering unisexuals are visited by flies only, cosexuals are visited by both flies and large-bodied bees later in the season. Bees avoid unisexuals because of their low density, small flowers, and limited pollen rewards relative to cosexuals (Case 2000). Contrasting foraging patterns of bees and flies have important consequences for mating patterns, particularly given the difference in ramet production between sexual systems. Extensive clonality in self-compatible cosexual plants visited by promiscuous pollinators can result in selfing through geitonogamy (Barrett 1984, Eckert 2000). Constraints imposed on cosexuals by selection to avoid geitonogamy are relieved for unisexuals, permitting ramification with little consequence to the mating system. Interactions between resources, pollination and mating are likely commonplace in plant reproduction, and highlight the need to consider both abiotic and biotic factors in ecological studies of gender variation.

#### *Gender-specific resource allocation and physiology*

The observed pattern of ecological segregation in sympatry suggests that plants in monomorphic and dimorphic populations of *W. dioica* should differ with respect to mechanisms governing the acquisition and allocation of resources. Although females, males, and cosexuals differed in both morphological (Fig. 6) and physiological (Table 2) responses to resource availability, total resource acquisition by the three sexes is likely to be comparable, as we detected no differences in total ramet biomass among them (Fig. 5). However, with increasing size, unisexuals allocated significantly less biomass above ground than cosexuals. Differences in allocation allometries resulted in cosexuals that were significantly taller, with greater leaf area, and larger flowers but smaller corms and fewer roots than unisexuals. These contrasting phenotypes are expected given the observed difference in microsite water availability between the sexual systems, and are consistent with earlier studies on biomass allocation patterns of plants under water stress (Gutschick 1981, Zimmerman and Lechowicz 1982, Schultze et al. 1987, van den Boogaard et al. 1996a, b). The increased storage of starch and water resources (as indicated by larger corm size) coupled with enhanced potential for water and

nutrient acquisition (from higher root production), reduced potential for transpirational water loss (lower leaf area), and smaller resource sinks (stems and flowers) of unisexuals may ameliorate the effects of reduced water availability in sites occupied by dimorphic populations.

Some aboveground size-related traits of cosexuals and unisexuals showed almost no overlap in their distributions (e.g., Fig. 6A, B, and D). Measures of stem height, leaf area, flower mass, and flower size were consistently larger, and with the exception of flower size, these traits varied positively with total ramet biomass among cosexual plants. In contrast, males and females maintained short stature, smaller leaf area, and smaller flowers regardless of changes in mass. If these patterns reflect selection for optimal resource allocation, then fitness gain when female and male functions are combined must increase with aboveground size. Although flower number per ramet did not differ among the sexes, both males and cosexuals exhibited significant positive relations between size and flower production (Fig. 6C). The absence of this relation among female plants suggests that flower number may contribute more to male than to female fitness gain in *W. dioica*, as suggested by Vaughton and Ramsey (1998). The distinctive patterns of biomass partitioning observed between sexual systems may reflect dissimilar evolutionary options for allocating resources by individuals with combined vs. separate sexes.

How did the two sexual systems respond to year-to-year changes in resource availability? This question is relevant to the ecology of *W. dioica* because this species is particularly noticeable after fire (Macfarlane 1980, Pate and Dixon 1982). Fire in Australian plant communities is known to increase water, nutrient, and light availability, and decrease competition (reviewed in Gill 1981). Although we can assume that conditions were better for all geophytes in 1995 than 1996 as a result of the fire, we might predict that unisexuals of *W. dioica*, with their probable greater capacity for belowground resource acquisition, would take better advantage of the increase in resources following the 1994–1995 fire than cosexuals. Plants of both sexual systems produced more flowers in 1995 compared with 1996; this difference was significantly greater for unisexuals (Fig. 3C), an effect that was augmented by a greater number of reproductive ramets per unisexual genet in 1995 than in 1996 (Fig. 3B). As discussed above, this increase in total flower number would be less constrained for separate sexes because of the absence of mating costs associated with geitonogamy.

While female and cosexual plants produced similar proportions of ovuliferous flowers in 1995 and 1996, male plants showed a dramatic reduction in allocation to female function, presumably precipitated by lowered resource availability in 1996. This response is consistent with several studies of gender dimorphic species demonstrating reduced fruit production by hermaph-

rodites in more resource-limited conditions (Webb 1979, Arroyo and Squeo 1990, Delph 1990b, Barrett 1992, Wolfe and Shmida 1997, Ashman 1999). While in *W. dioica* male plants in dimorphic populations and cosexual plants in monomorphic populations are both functionally hermaphroditic, their differential response to stress in terms of female allocation reflects their respective gender roles within the two sexual systems. Reductions in seed production by male plants are not as costly as they would be to cosexual plants because males gain the majority of their fitness through male function (see Delph and Lloyd 1991). In contrast, cosexuals obtain, on average, half of their reproductive fitness through seed, hence relative allocation to female vs. male function by cosexuals should not be reduced in the face of resource limitation.

Dawson and Geber (1999) have argued that trade-offs between resource acquisition and allocation may be central to habitat specialization in plants. A trade-off between resource acquisition and reproductive allocation may also contribute to selection for gender specialization in low resource environments. If high belowground allocation is required to obtain sufficient resources from the soil, this necessarily reduces resource availability for reproduction. Therefore, plants with lower reproductive expenditure, such as unisexuals of *W. dioica*, could potentially enjoy a fitness advantage over cosexuals in low resource environments. In unisexual plants of *W. dioica*, reproductive allometries and correlations among allocation traits indicate strong resource trade-offs between belowground vs. aboveground allocation (Table 3A). Thus the existence of this trade-off likely underlies the observed association between poor microsites and gender dimorphism. A trade-off between belowground and aboveground biomass was not evident for cosexual plants (Table 3B). This may be a consequence of limited residual variation in components of allocation once total mass was taken into account (see Fig. 5), which may be because plant size is a more important determinant of whole plant allocation patterns in cosexuals compared with unisexuals. It is also possible that aboveground allocation by cosexuals trades off with some other trait or currency that we did not measure (see Delph and Meagher 1995). Alternatively, trade-offs may be more apparent under low resource conditions (Primack et al. 1994) if stress results in more limited variation in acquisition compared with allocation (van Noordwijk and de Jong 1986).

Our expectations that plants in wetter microsites with greater reproductive expenditure would have higher leaf N and lower WUE (water use efficiency) were not met. On a per mass basis, leaf N content was almost twice as high in females and males than in cosexuals. During both July and August, females and males were equally or less water use efficient than cosexuals despite their occurrence in significantly drier microsites. The higher leaf N content and lower WUE of unisexuals

suggest that females and males have a higher photosynthetic capacity than cosexuals, although the greater leaf area (i.e., greater photosynthetic surface area) of cosexuals may enhance total photosynthetic output at the plant level (Percy et al. 1987, Schultze et al. 1987). The strong positive correlations between vegetative and reproductive mass in all three sexes suggest that this may be the case: increased leaf area enhances resource availability for reproduction. Females and males were more water use efficient during flowering than fruiting, which is consistent with the increase in soil moisture from July to August (Fig. 4). This suggests that rates of photosynthesis and resource acquisition during this period increase and this increased stomatal conductance would support the costs of seed production.

Water limitation should favor increased WUE in general (Ehleringer 1993, Dudley 1996), and is often associated with reduced photosynthetic activity and lower leaf N content (Percy et al. 1987, Donovan and Ehleringer 1994, but see Dudley 1996). Unexpectedly, the patterns of WUE and leaf N content we measured in *W. dioica* contradict these predictions. However, biomass allocation patterns may influence both WUE and photosynthesis independently of, or in concert with, environmental factors, and may partly explain our results. Reduced leaf area and increased belowground allocation, as we observed among unisexuals, are common responses of plants to limited water supply (Schultze et al. 1987, Dudley 1996). However, roots and storage organs, which are critical for obtaining, storing, and distributing resources when limiting, are costly in terms of carbon such that plants may be required to maintain high levels of photosynthesis at the cost of WUE by keeping stomata open (Chapin et al. 1987).

Plants with substantial aboveground allocation, particularly those with high leaf area, may use stomatal closure to maintain leaf turgor over a larger surface area (Schultze et al. 1987, Donovan and Ehleringer 1994, Dudley 1996). This could explain the higher WUE of cosexuals despite their occurrence in wetter microsites. However, stomatal closure may reduce photosynthetic rates per unit of leaf area by reducing the diffusion of CO<sub>2</sub> into the leaf. A recent study of experimental populations of monomorphic *Cakile edentula* (Brassicaceae) supports this argument (Dudley 1996). Plants grown in dry conditions produced smaller leaves, but had significantly lower WUE, higher rates of stomatal conductance, and higher rates of photosynthesis than plants grown in wet conditions, despite positive selection for both increased leaf size and WUE and negative selection for stomatal conductance in dry environments (Dudley 1996). In *W. dioica*, the lower leaf N of cosexuals may reflect diminishing returns on the investment of N in photosynthetic machinery when internal CO<sub>2</sub> concentrations are likely to be relatively lower because of reduced stomatal conductance (Chap-

in et al. 1987). We did find a negative correlation between leaf area and leaf N content per unit mass, but only among female plants.

Monomorphic and dimorphic sexual systems of *W. dioica* clearly differ in the manner in which they acquire and allocate resources. The question remains as to whether these gender-related differences in biomass allocation and physiology represent contrasting adaptive strategies for dealing with more or less stressful conditions, or plastic responses to microsite variation in soil moisture availability. Manipulative experiments to discern these effects would be difficult in *W. dioica*. However, studies of geographical variation provide some insight into this issue. The patterns of biomass allocation we observed in sympatry were apparent across 12 populations of *W. dioica* along a latitudinal precipitation gradient in southwestern Australia (Case 2000). Although proportional allocation patterns did vary with changes in precipitation, plants in the dimorphic populations had consistently greater below-ground mass than plants in the monomorphic populations. This suggests that the sexual systems of *W. dioica* likely employ different allocation strategies despite some degree of plasticity.

Charlesworth (1999) emphasized the need for connections between theoretical models on the evolution of gender dimorphism with the common observation that gender dimorphic plants often occur in stressful conditions and resource-limited sites. Contrasting resource allocation patterns and habitat preferences have been shown for females and males of dioecious plant species, and several studies have also demonstrated physiological differences between the sexes (reviewed in Dawson and Geber 1999, Delph 1999). However, data on the morphology, physiology, and habitat specialization of closely related taxa with combined and separate sexes are almost nonexistent (see Costich 1995). We have demonstrated that the sexual systems of *W. dioica* differ markedly in demography, biomass allocation, and physiology in sympatry, which may represent gender-specific strategies for dealing with limited water availability. Our results emphasize the importance of habitat characteristics for the maintenance of gender variation, and point to the need to incorporate ecological context into theoretical models.

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