

FLORAL BIOLOGY OF GENDER MONOMORPHISM AND DIMORPHISM IN *WURMBEA DIOICA* (COLCHICACEAE) IN WESTERN AUSTRALIA

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We compared the floral biology of female, male, and cosexual plants in sympatric populations of *Wurmbea dioica* (Colchicaceae) from Western Australia to assess evidence that changes in pollination biology accompanied a transition between monomorphic to dimorphic sexual systems in this species. In *W. dioica*, sex-specific differences in floral design and display, the quantity and quality of rewards, and reproductive phenology represent alternative strategies for pollinator attraction and the avoidance of self-pollination. Unisexual plants had smaller flowers containing less pollen than flowers of cosexuals; males and cosexuals produced nectar with higher sugar content than did females. These patterns were associated with differences in the composition and visitation rates of pollinators. Nectar-foraging flies visited all three sexes, but pollen-collecting bees visited only cosexuals. Fly-pollinated females achieved greater seed fertility relative to their bee-pollinated cosexual relatives, and males and cosexuals received longer and more frequent visits than did females. Although there were no differences in flower number among sexes, females maintained larger displays throughout flowering. In contrast, males and cosexuals staggered both floral anthesis and anther dehiscence as mechanisms for promoting enhanced pollen dispersal. We propose that sex-based differences in floral design and display in *W. dioica* have mediated a shift in the effectiveness of nectar-foraging insects as pollinators and that gender dimorphism enforces outcrossing under pollination conditions that would otherwise cause selfing. Differences in pollination biology probably also contribute to the maintenance of combined versus separate sexes in sympatric populations of *W. dioica* in Western Australia.

Keywords: evolution of dioecy, insect pollination, floral longevity, pollen presentation, stigma-anther separation, protogyny.

Introduction

The evolution of separate sexes has occurred repeatedly during the history of flowering plants in a wide variety of phylogenetic and life-history contexts and ecological conditions. Comparative studies have established associations between gender dimorphism and several morphological, ecological, and biogeographical features, including large plant size, perennality, woodiness, fleshy fruits, small inconspicuous flowers, generalist pollinators, aridity, island habitats, and tropical climates (Yampolsky and Yampolsky 1922; Bawa 1980; Thomson and Brunet 1990; Renner and Ricklefs 1995; Sakai and Weller 1999; Webb et al. 1999; Vamosi et al. 2003). No single causal agent can be invoked to explain the origin of gender dimorphism in flowering plants; however, two factors have most certainly played a role: the avoidance of inbreeding and the relative fertility of individuals with combined versus separate sexes (Lloyd 1982; Charlesworth 1999).

Pollination is an important factor in the evolution of sexual systems because pollinators govern mating opportunities within populations by mediating pollen dispersal within and between plants. Variation in rates of self-pollination and fe-

male and male reproductive success are strongly influenced by the quality of pollinator service (reviewed in Harder and Barrett 1996). Most animal-pollinated species with gender dimorphism are pollinated by “indiscriminant” or “promiscuous” pollen vectors, such as small bees and flies (Bawa and Opler 1975; Ganders 1978; Bawa 1980; Delph 1990; Weller and Sakai 1990; Delph and Lively 1992; Renner and Ricklefs 1995). In many cosexual species, these types of pollinators often provide inferior pollinator service relative to more specialized insects—transferring enough pollen to overcome pollen limitation of seed set but limiting the production of high-quality outcrossed offspring because of restricted pollen dispersal. If cosexual plants experience a reduction in the quality of pollinator service, resulting in increased selfing and inbreeding depression, selection for the separation of the sexes may be favored as an outcrossing mechanism (Ganders 1978; Bawa 1980; Lloyd 1982; Arroyo and Squeo 1990; Delph 1990; Thomson and Brunet 1990; Weller and Sakai 1990; Sakai and Weller 1999).

Evaluating hypotheses on the role of pollination biology as a selective force in the separation of the sexes is complicated by the fact that evolutionary transitions are difficult to study. That is, we are forced to compare situations where the evolution of gender dimorphism either has or has not been favored. As noted by Charlesworth (1999), conditions present in most monomorphic populations are unlikely to favor the evolution of gender dimorphism; otherwise, monomorphism would not be maintained. Conditions present in dimorphic

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populations may have changed since dimorphism originated, possibly providing misleading clues about which factors were important. Comparisons of closely related taxa differing in sexual system may provide insight into this issue because the transition between monomorphism and dimorphism is more likely to have occurred relatively recently. Thus, more of the morphological and ecological differences between the sexual systems are likely to be functionally associated with selection for separate sexes.

Wurmbea dioica ssp. *alba* is a diminutive, self-compatible, insect-pollinated geophyte that is widespread in southwestern Australia. Populations are either monomorphic (containing co-sexuals) or dimorphic (containing females and males) for gender, with contrasting sexual systems occurring sympatrically at several locations along the Darling Escarpment near Perth (Barrett 1992; Case and Barrett 2001). Sympatric sites provide opportunities to compare monomorphism and dimorphism independently of geographical variation in environmental conditions and community composition, particularly pollinator pools, which often confound comparative studies of allopatric populations. To investigate the role of pollination biology in selection for gender dimorphism, we compared sympatric populations of the sexual systems of *W. dioica* ssp. *alba* for (1) strategies of pollinator attraction and pollen dispersal, (2) the potential for self-pollination, and (3) the relative fertilities of unisexual and cosexual plants. This was accomplished by contrasting females, males, and cosexuals with respect to the following attributes: floral display, the quantity and quality of floral rewards, reproductive phenology, the frequency and duration of insect visitation, rates of pollen removal and deposition, and female fertility (fruit and seed set). We also conducted field experiments to assess the effects of pollinator exclusion on reproductive phenology and the supplementation of cross- and self-pollen on female fertility.

Methods

Study Species and Site

Wurmbea dioica ssp. *alba* plants are composed of an underground corm and an annual shoot, consisting of one basal and two cauline linear leaves and an erect cymose inflorescence (Macfarlane 1980). All plants in monomorphic populations and the majority of plants in dimorphic populations produce only one flowering shoot per reproductive season; however, unisexuals can produce multiple reproductive ramets (up to 16; Case 2000). Inflorescences contain an average of 2–3 flowers (range 1–6) that can be pistillate, staminate, or hermaphroditic (perfect). Anthesis is acropetal, and perfect flowers are protogynous. 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, females produce all pistillate flowers, while males produce varying proportions of staminate and perfect flowers (Barrett 1992), the proportion of perfect flowers averaging ca. 25% for all males. Here 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.

In sympatry, the sexual systems are phenologically segregated with respect to flowering (Case and Barrett 2001) but not seed dispersal. Plants in dimorphic populations flower from late June to late July, while plants in monomorphic populations flower from early August to the start of September (Case and Barrett 2001). Fruits of both sexual systems mature in late September and October, with seeds shaken from the dry dehiscent capsules by wind. Although pollination and mating take place at different times, our observations indicate that the insect fauna is qualitatively similar during each flowering time. Thus, differences in pollination biology between the sexual systems are not simply a consequence of a shift in flowering time resulting in changes to the composition of the pollinator pool.

Our study was conducted July–September 1996 at a sympatric site near Lesmurdie, Western Australia, ca. 40 km east of Perth (32°00′27″S, 116°01′42″E, 150 m elevation). The site is characterized by open scrub composed of native vegetation (*Xanthorrhoea*, *Eucalyptus*, *Acacia*, and *Melaleuca* spp.) growing in rocky clay loam over granite on the southwest face of the Darling Escarpment. Populations of each sexual system consisted of 8000–10,000 plants distributed over ca. 2 ha. The frequency of female plants in the dimorphic population was 0.40.

Pollinator Attraction and Reward

We assessed variation between the sexual systems and among sexes in three components of floral display: flower number, flower size, and flower spacing. We measured the total number of flowers per inflorescence, tepal length, and the distance between flowers on each inflorescence for 30–55 plants of each sex. We also measured the distance between the tips of opposing tepals (flower diameter) to assess variation in apparent flower size resulting from differences in the degree of tepal spreading.

To determine pollen size and number, we collected inflorescences from males and cosexuals prior to anthesis ($n = 71$ –87 flowers per sex), maintained them indoors in water-filled microfuge tubes, and transferred newly dehiscent anthers to 70% ethanol. We counted and measured pollen using a Particle Data Elzone 282PC particle counter (Micromeritics, Norcross, Ga.). Following pollen counts, we measured the length of each anther and the size of each flower to obtain correlates of pollen production to be used for assessing pollen removal. We measured tepal lengths using an ocular micrometer under a dissecting microscope, while anther sac lengths were measured using a compound microscope (Zeiss Axioplan IS1988) connected to a digital imaging system (Empix Imaging 1995).

Because *W. dioica* ssp. *alba* plants produce minute amounts of nectar, we obtained one nectar sample for each sex by bulking nectar from 40 flowers per sex. We collected inflorescences of 20 two-flowered plants per sex prior to anthesis and maintained them indoors in water, allowing nectar to accumulate for 24 h. We collected nectar from flowers of each sex morph into 1- μ L capillary tubes (total nectar volume from 40 flowers was ca. 1 μ L). Nectar sugars were analyzed with a Waters HPLC system (600E pump, 717 autoinjector, 410 refractive index detector, and 996 photodiode-array

detector; Milford, Mass.). A mobile phase of 5 mM H₂SO₄ at a flow rate of 0.5 mL/min was used to separate the sugars on a 300 × 7.8-mm Bio-Rad HPX-87H (Hercules, Calif.), with the column temperature of 45°C.

We assessed differences between the sexual systems and among sexes within sexual system using nested ANOVA and ANCOVA in JMP (version 3.2.2; SAS Institute 1997). Because there are important mating system consequences of bearing perfect flowers, we treated males with and without perfect flowers (“fruiting” vs. “nonfruiting” males) separately in all analyses and used *F*-tests on least square means to determine whether these two classes of males were significantly different for each trait. We combined these classes for data presentation when they were not significantly different, and we conducted planned pairwise (Tukey’s) comparisons on least square means for each sex when there were significant differences indicated by the ANCOVAs. We used flower size and number as covariates where appropriate. Unless otherwise indicated, all continuous response variables and covariates were natural log transformed to meet assumptions of normality (verified using Shapiro-Wilk tests in JMP), and main effects were considered fixed effects.

Phenology within Flowers and Inflorescences

We investigated variation among sexes in the timing of anthesis within inflorescences and of anther dehiscence within flowers and the effect of pollinator exclusion on phenological patterns. For each sexual system, we marked all individuals within a 3-m² plot ($n = 45$ – 60 per sexual system) prior to anthesis; half of the individuals in each plot were bagged with fine mesh to exclude floral visitors, while the rest were left open. We surveyed the plot every other day and recorded the state of each flower (in bud, open, or senescent) and the state of each anther (undehisced, dehiscing, or empty). Esterase assays (Kearns and Inoue 1993) indicated that stigmas are receptive just before anthesis until flowers begin to senesce (Case 2000). From these survey data, we calculated the following traits for each individual: total time spent flowering, floral longevity, time between anthesis of successive flowers, time from anthesis until the first anther dehiscence (degree of protogyny), and the time until all anthers had dehiscence (rate of pollen metering). We performed nested ANCOVAs to assess differences between sexual systems and among the sexes, and between bagged and unbagged plants, adjusting for variation in total flower number.

Insect Visitation

We established several plots per sexual system for observations of floral visitation by insects; plots were at least 1.5 m² and were separated by at least 50 m. Observation periods were 15 min in length and were taken over a period of 19–20 d per sexual system (dimorphic: June 26–July 13; monomorphic: Aug. 12–Sept. 1). We observed floral visits between 10:00 AM and 5:00 PM each day. For each visit, we recorded the identity of the insect, visit duration, and the sex and total number of flowers visited. From this information, we determined the frequency of visits to each sex by each insect, the length of each visit, and the effect of flower number on visi-

tion rate. We also kept track of individual plants and insects within each observation period and determined the number of multiple visits to plants of each sex. We were particularly interested in visit duration per flower and the frequency of multiple visits by individual insects because both of these are likely to promote self-pollination.

Pollen Removal and Deposition

To determine rates of pollen removal and deposition to individual flowers, we marked 50 plants of each sex prior to anthesis, surveyed each plant daily, and recorded the first day of anthesis and the first day of pollen presentation for each flower. We considered anthesis the first possible day for pollen deposition, and we considered anther dehiscence the first day that pollen removal could occur. At 2-d intervals (i.e., 1, 3, 5, 7, and 9 d) following anthesis or anther dehiscence, we preserved stigmas, anthers, and the remaining flower parts in 70% ethanol, each in separate tubes; anthers were first collected into dry tubes until all anthers had dehiscence, and then ethanol was added. Plants showing evidence of predation were replaced when possible.

We estimated the amount of pollen removed per flower as the total amount produced less the amount of pollen remaining in the flower. We measured pollen remaining in flowers after each time interval as for pollen production. We estimated total pollen production as the total anther sac length, which is a reliable predictor of total pollen production ($R = 0.81$, $P < 0.0001$). We calculated the rate of pollen removal by plotting the proportion of pollen removed per flower against the number of days of pollen presentation. We measured stigmatic pollen loads by counting pollen on stigmas under UV light using a compound microscope (Zeiss Axioplan IS1988). We calculated the rate of pollen deposition per flower per day as the total number of grains deposited per day after anthesis.

We used nested ANCOVA to assess the effect of sexual system, plant sex, the number of days of visitation, and flower size, number, and position (numbered from the base of the inflorescence) on the proportion of pollen removed per flower and the total number of grains deposited per flower. We excluded females from the analysis of pollen removal; thus, the sex effect tests for differences in pollen removed from staminate versus perfect flowers within each sexual system. To equalize variances, we logit transformed data on the proportion of pollen removed.

Female Fertility

We estimated ovule number by dissecting carpels of 50 preserved flowers per sex. During flowering, one flower per plant was either unmanipulated ($n = 60$ per sex), supplemented with cross-pollen ($n = 50$ plants per sex), or self-pollinated ($n = 30$ plants per polleniferous morph). Comparisons between manipulated (both self- and cross-pollinated) and unmanipulated plants were made to assess the potential for inbreeding depression and the degree of pollen limitation, respectively. We scored fruit set as a binary character (successful vs. unsuccessful) and analyzed the variation using logistic regression.

Table 1
ANCOVA of Floral Display in Monomorphic and Dimorphic Sexual Systems of *Wurmbea dioica* at a Sympatric Site near Perth, Western Australia

Source	Flower number	Flower spacing	Tepal length	Flower diameter
Sexual system	2.55	95.3 ^{****}	122 ^{****}	13.0 ^{***}
Sex (sexual system)	1.52	2.10	5.0 ^{**}	7.6 ^{***}
Flower number	...	0.99	4.37 [*]	0.0041
Tepal length	22.3 ^{****}

Note. We assessed variation among sexual systems ($df = 1$) and among sexes ($df = 2$) for flower number, tepal length, flower diameter, and the distance between flowers. Flower number ($df = 1$) and tepal length ($df = 1$) were included as covariates where appropriate; all variables were natural log transformed for analysis. F -values are shown; P -values are indicated by superscripts.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

**** $P < 0.0001$.

Results

Floral Design and Display

The number of flowers per inflorescence did not differ significantly among plants of the three sexes; however, other aspects of floral design and display distinguished the two sexual systems (table 1; fig. 1; fig. 2A, 2B). In monomorphic populations, the styles of perfect flowers were erect with capitate stigmas, whereas in dimorphic populations, the styles of both perfect and pistillate flowers were recurved with elongate stigmas (fig. 1). Flowers of cosexual plants had longer tepals (fig. 2B), and flowers were farther apart on the inflorescence (distance between flowers = 10.8 ± 0.44 mm) than those of unisexuals (females: distance = 4.66 ± 0.26 mm; males: distance = 4.73 ± 0.30 mm). Within the dimorphic population, the tepals of males were significantly longer than those of females but only by an average of 0.67 mm (fig. 2B). However, all three sexes differed substantially with respect to flower diameter (apparent flower size), even after the effect of tepal length was taken into account (table 1). This is because variation in flower diameter is influenced by both tepal length and the angle between the tepals and the floral axis (fig. 1). Female flowers opened to only $58.9\% \pm 1.8\%$ of their maximum diameter (twice the tepal length), substantially less than those of males ($79.8\% \pm 4.3\%$) and cosexuals ($88.1\% \pm 1.6\%$; males and cosexuals did not differ, Tukey's HSD $P > 0.05$). Although there was a significant negative relation between flower number and tepal length, there was no effect of inflorescence size on flower diameter (table 1). Pair-

wise comparisons of least square means indicated that males with or without perfect flowers did not differ with respect to any of these traits (all $P > 0.10$).

Floral Rewards

The sexual systems differed significantly in both pollen production and nectar quality. Cosexual plants produced two to three times more pollen per flower ($F_{1,153} = 87.5$, $P < 0.0001$; fig. 2C) and smaller pollen grains ($F_{1,150} = 41.5$, $P < 0.0001$; pollen length = 19.5 ± 0.19 μ m) than did male plants (pollen length = 21.7 ± 0.22 μ m). Neither flower sex (fig. 2C, circles; $F_{1,86} = 0.04$, $P = 0.84$) nor flower size ($R = 0.11$, $P = 0.31$, $n = 87$) affected pollen production by males. In contrast, staminate flowers of cosexuals (fig. 2C, closed triangles) contained significantly less pollen than did their perfect flowers (fig. 2C, open triangles; $F_{1,70} = 9.76$, $P < 0.0026$), and pollen production was positively related to flower size ($R = 0.34$, $P = 0.0034$, $n = 71$). The polleniferous morphs also differed in the relation between pollen size and pollen number, which was negative among males ($R = -0.21$, $P = 0.05$, $n = 84$) and significantly positive among cosexuals ($R = 0.29$, $P = 0.0014$, $n = 71$).

Male plants produced nectar with a higher concentration of all sugars (table 2). Sucrose content was higher for females than cosexuals, while glucose and fructose concentrations were higher for cosexuals. With only one sample per sex, we were unable to assess differences in nectar characters statistically, which limits our ability to generalize these patterns to a larger population. However, the precision of HPLC and the

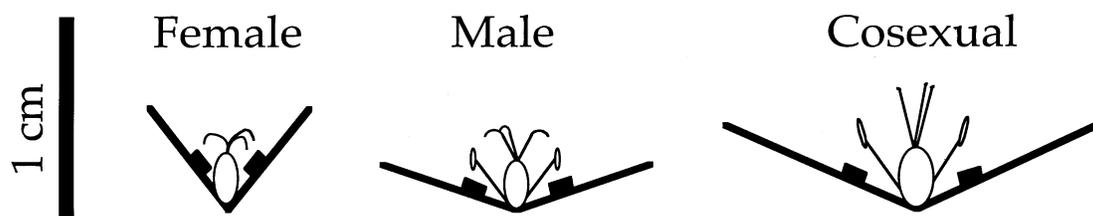


Fig. 1 Longitudinal sections of typical pistillate and perfect flowers of female, male, and cosexual plants of *Wurmbea dioica* ssp. *alba*. Tepals, stamens, and pistils are drawn to scale. Black boxes on tepals represent transverse nectary bands; their positions are to scale, and their heights are exaggerated. The angle of opposing tepals represents the source of variation in flower diameter.

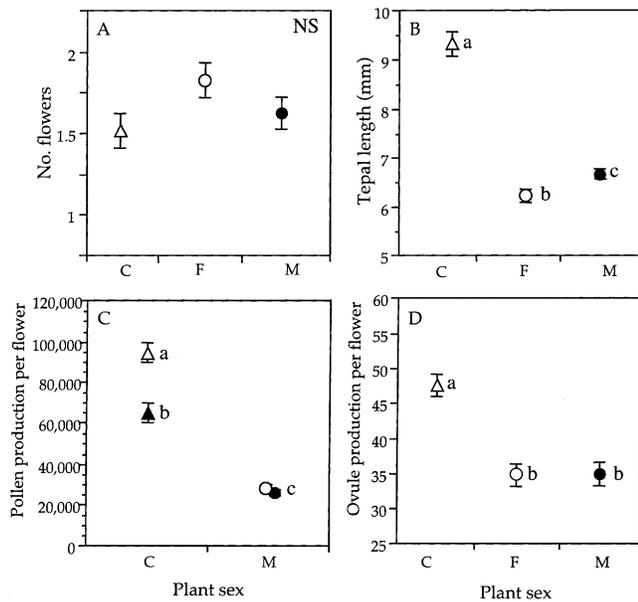


Fig. 2 Aspects of floral design and display of plants in the monomorphic and dimorphic sexual systems of *Wurmbea dioica* at a sympatric site near Perth, Western Australia. Means \pm 1 SE for the number of flowers per inflorescence (A), tepal length (B), pollen (C), and ovule production per flower (D) are shown for females (F), males (M), and cosexuales (C). Letters indicate means that were not significantly different at $P = 0.05$ (Tukey's tests). Closed symbols in C represent staminate flowers; open symbols are perfect flowers.

pronounced differences observed provide confidence in the observed sex differences among the specific samples analyzed in this study.

Reproductive Phenology

Patterns of reproductive phenology varied among sexes and between pollinator exclusion treatments (table 3). When insect visitation was permitted, female plants spent less time in flower than did males or cosexuales because of significant differences in both the time between the anthesis of successive flowers (fig. 3) and individual floral longevities (fig. 4). Flowers of females were shorter lived and opened more synchronously compared with males; cosexuales were statistically intermediate with respect to both traits. When pollinators were excluded, differences in flowering duration were greatly reduced because of a sex-specific effect of the treatment on individual floral longevity (table 3). Pollinator exclusion increased the longevities of flowers on female and cosexual plants but shortened those on male plants, particularly of staminate flowers (fig. 4). Total flower number per inflorescence was positively related to total flowering duration and individual floral longevity but had no effect on patterns of anthesis or anther dehiscence.

The polleniferous morphs differed significantly in both the time from anthesis to anther dehiscence (table 3) and the rate of anther dehiscence within flowers. Cosexuales took nearly twice as long to begin pollen presentation as males

(4.4 ± 0.21 d vs. 2.3 ± 0.27 d, respectively); once pollen was presented, anther dehiscence in males occurred at a lower rate (fig. 3, hatched bars). We detected no difference between staminate and perfect flowers within sexual systems (table 3). Compared with cosexuales, flowers on male plants (1) had fewer dehiscent anthers on the first day of pollen presentation (2.16 ± 0.21 vs. 3.07 ± 0.17 ; Mann-Whitney U two-sample test: $Z = -3.38$, $P = 0.0007$), (2) opened fewer anthers on each successive day during the male phase (1.62 ± 0.21 vs. 2.15 ± 0.20 anthers per day, $Z = -2.76$, $P = 0.006$), and (3) took longer to dehisce all six anthers within each flower (5.43 ± 0.38 d vs. 4.40 ± 0.28 d, $Z = 2.11$, $P = 0.034$).

Several differences in stamen traits between males and cosexuales were observed. Some male plants reclosed their previously dehiscent anthers during rainy and windy weather. Only anthers containing a substantial amount of pollen reclosed, typically those that had been open for fewer than 3 d. We found no evidence of reversible anther dehiscence in cosexuales, and this may be related to observed differences in stamen morphology. The anthers of male plants are fully versatile, with pollen readily dislodged on shaking, whereas the anthers of cosexuales are fixed and pollen must be removed by contact. Preliminary observations of stamen structure indicate (1) a high degree of lignification of the anthers of cosexuales that is absent in males, (2) broader filaments at the point of anther attachment in cosexuales, and (3) a greater number of xylem elements in the filaments of males. These traits likely contribute to the versatility of anthers as well as the capacity for anther reclosure, the latter of which probably involves rehydration (Edwards and Jordan 1992).

Insect Visitation

We recorded a total of 877 insect visits in 27.5 h of observation: 275 to unisexuals and 602 to cosexuales. Flies of various sizes were the most frequent visitors to plants of both sexual systems (fig. 5), the most common being small dance flies (Empididae) and dark-winged fungus gnats (Sciaridae). All flies foraged for nectar only. Nonnative honeybees collected pollen from cosexual plants, but we never observed them visiting unisexual plants. Visits by nectar-foraging ants and butterflies composed a smaller fraction of the total visits to plants; beetles and katydids were occasionally observed eating various parts of flowers. Of all visitors, bees and flies are the most important pollinators of *Wurmbea dioica* ssp. *alba*.

The rate and the duration of fly visits was greatest for cosexuales and was higher for males than for females; however,

Table 2

Concentration of Sugars in Nectar Produced by Females, Males, and Cosexuales of *Wurmbea dioica*

Sex	Sucrose	Glucose	Fructose
Female	7.4	14.9	15.6
Male	43.7	188.6	157.6
Cosexual	2.0	34.9	35.7

Note. We performed analyses on bulk samples of nectar collected from 40 flowers per sex. All concentrations are in $\mu\text{mol/mL}$ and are corrected for injection volume.

Table 3

ANCOVA of Phenological Patterns in Plants from Monomorphic and Dimorphic Sexual Systems of *Wurmbea dioica* at a Sympatric Site near Perth, Western Australia

Source	Floral longevity	Time between flowers	Time to pollen presentation
Sexual system	0.22	4.27*	34.5****
Sex (sexual system)	18.7 ^{a,****}	14.8 ^{a,****}	0.53 ^b
Treatment	12.1 ^{***}	1.35	1.59
Treatment × sexual system	4.86*	<0.01	1.71
Treatment × sex (sexual system)	12.8****	1.95	1.45
Flower number	4.32*	0.01	<0.01

Note. We assessed variation between sexual systems ($df = 1$), variation among sexes within sexual systems ($df = 1-2$), and the effect of pollinator exclusion treatment ($df = 1$) on the longevity of individual flowers, the time between anthesis of adjacent flowers, and the time from anthesis until pollen presentation. We excluded females from the analysis of time to pollen presentation and used flower number as a covariate in each analysis; all traits were measured in days. F -values are shown; P -values are indicated by superscripts.

^a $df = 1$: sex effect compares traits of female and male plants in dimorphic populations.

^b $df = 2$: females excluded, sex effect compares staminate and perfect flowers of cosexual and male plants.

* $P < 0.05$.

*** $P < 0.001$.

**** $P < 0.0001$.

unisexuals were more likely than cosexuals to receive multiple visits by individual insects within a given observation period (table 4). Total flower number per inflorescence had no effect on visitation by either flies ($F_{1,365} = 0.30$, $P = 0.58$) or bees ($F_{1,214} = 0.0002$, $P = 0.98$), and within the monomorphic population, there was no difference in bee versus fly visitation rates ($F_{1,214} = 1.94$, $P = 0.16$). In contrast, visitation rate and the duration of visits to each sexual system varied significantly with insect body size (fig. 6). We classified flies into three size categories based on their body length, all of which were smaller than honeybees: small = <3 mm, medium = 3–5 mm, large = 5–10 mm. Smaller insects were the predominant visitors to unisexual plants, whereas larger insects primarily visited cosexuals, and visit duration decreased with body size.

Pollen Removal and Deposition

We detected significant differences between the sexual systems in the proportion of pollen removed and the number of pollen grains deposited per flower (table 5). Although the rate of pollen removal over the 9-d sampling period was equivalent between males and cosexuals, a greater proportion of total pollen was removed from the flowers of cosexuals because more pollen was removed on day 1 (fig. 7A). This pattern likely occurred because flowers of cosexuals had more dehiscent anthers on the first day of pollen presentation; thus, a greater proportion of total pollen was available for removal. By the end of the sampling period (9 d after pollen presentation), 92.8% (± 2.4) of pollen had been removed

from the flowers of cosexuals, and 75.8% (± 5.4) was removed from males. Total flower number had no effect on pollen removal (table 5). The positive relation between flower size and pollen removal reflects between-group rather than within-group differences; flower size had no effect on pollen removal within sexual systems (fig. 7B). We detected no differences in pollen removal from plants with or without perfect flowers (table 5).

Rates of pollen deposition to cosexuals and females were not significantly different and were higher than deposition to male plants (table 5; fig. 8). Flower number, but not flower size, varied positively with deposition to individual flowers (table 5). Increased deposition with flower number was significant among females ($F_{1,48} = 10.6$, $P = 0.002$) and cosexuals ($F_{1,72} = 9.02$, $P = 0.036$) but not significant among males ($F_{1,19} = 0.05$, $P = 0.83$).

Female Fertility

We detected significant differences between the sexual systems in ovule number and seed set but not fruit set (table 6). Cosexuals produced more ovules than did females or fruiting males, and unisexuals did not differ in the number of ovules per flower (fig. 2D). Hence, there was no evidence of reproductive compensation associated with male sterility. Because females produced more ovuliferous flowers, total ovules per plant was greater for females than fruiting males (51.1 ± 5.7 vs. 29.1 ± 5.3 , respectively; $F_{1,86} = 8.11$, $P = 0.006$). Open-pollinated fruit and seed set were relatively low for all three ovuliferous morphs, but there was no evidence that plants

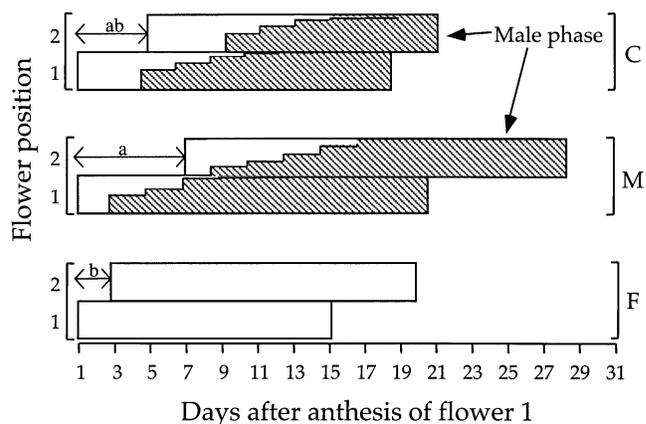


Fig. 3 Schematic showing patterns of floral longevity, anthesis, and anther dehiscence of female (F), male (M), and cosexual (C) plants of *Wurmbea dioica* at a sympatric site near Perth, Western Australia. Mean trait values for two-flowered plants of each sex are shown. Each flower is represented by a box. The length of each box represents mean floral longevity. The position of the second box along the horizontal axis represents the mean time between the anthesis of flower 1 and flower 2; letters on double-headed arrows denote significant differences in time to anthesis of flower 2 (Tukey's tests). The position of the hatched bars along the horizontal axis represents the time between anthesis and anther dehiscence, and the height of the hatched bars relative to the total height of the box represents the proportion of anthers dehiscing at each 2-d interval.

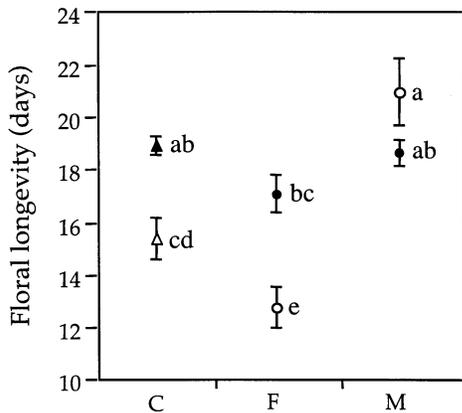


Fig. 4 The effect of pollinator exclusion on floral longevity of female (F), male (M), and cosexual (C) plants of *Wurmbea dioica* at a sympatric site near Perth, Western Australia. Means \pm 1 SE are shown where pollinators were (closed symbols) or were not excluded (open symbols). Letters indicate means that were not significantly different at $P = 0.05$ (Tukey's tests).

were pollen limited (fig. 9). Percentage fruit set of open- and cross-pollinated plants ranged from ca. 35% among fruiting males to an average of 60% among females. Seed to ovule ratios ranged from 0.05 to 0.07 for cosexuals, 0.14 to 0.16 for fruiting males, and 0.24 to 0.25 for females.

Pollination treatment had no effect on fruit or seed set, and the number of seeds per fruit following self-pollination was comparable to that of other treatments (cosexuals: 4.1 ± 1.1 seeds; males: 4.5 ± 3.4 seeds). Predation during both flowering and fruiting reduced sample sizes for each group but particularly for cross- and self-pollinated males ($n = 11$ and 6, respectively; all other $n = 25$ –50 per sex per treatment).

Discussion

The unusual occurrence of contrasting sexual systems in *Wurmbea dioica* ssp. *alba* has enabled us to make the first detailed comparison of the floral biology of gender monomorphism and dimorphism in sympatry. Our investigations revealed striking differences in reproductive traits and pollination biology between monomorphic and dimorphic sexual systems. We next interpret the functional consequences of this variation for mating and fertility and discuss how changes in the pollination biology of populations may have led to the evolution of separate sexes from combined sexes in *W. dioica*.

Gender-Specific Strategies for Attracting Pollinators

In general, insect pollinators prefer large flowers and displays (Bell 1985; Galen and Newport 1988; Eckhart 1991) because both are typically correlated with higher levels of reward (Ashman and Stanton 1991; Cohen and Schmida 1993; Galen 1996). The sexual systems of *W. dioica* ssp. *alba* exhibit contrasting strategies for attracting floral visitors, with sex-specific patterns of pollen production, nectar quality, and particularly flower size but not flower number.

Cosexuals had significantly larger flowers than did unisexuals (figs. 1, 2B), and larger flower sizes were associated with higher pollen production. The association between flower size and pollen production in the monomorphic population reflects the fact that staminate flowers of cosexuals are smaller and contain less pollen than do perfect flowers, likely because they are borne by small individuals of lower resource status. In contrast, there was no effect of flower sex or flower size on pollen production among male plants and no difference in flower size between perfect and staminate flowers of males.

Honeybees did not visit or even approach plants in the dimorphic population, probably because of a combination of low pollen availability, smaller flowers, and low population density (Case and Barrett 2001). Other studies of dimorphic species have demonstrated that pollen-collecting insects strongly discriminate against female plants but that nectar foragers are often less biased (Kay et al. 1984; Eckhart 1991; Delph and Lively 1992; Vaughton and Ramsey 1998). Therefore, although flower size and pollen production may be important components of pollinator attraction by cosexuals of *W. dioica* ssp. *alba*, these factors appear to be less important for females and males. Honeybees were introduced into Australia ca. 150 yr ago and cannot have influenced the origin of sexual systems in *W. dioica* ssp. *alba*. However, honeybees have likely displaced species of native Australian bees as floral visitors (reviewed in Paton 1993). Native Australian bees collect both pollen and nectar, although most are solitary and somewhat smaller than honeybees.

Flowers of male plants were slightly larger than those of females but by less than 1 mm per tepal. In contrast, the sexual morphs differed substantially in effective flower diameter and nectar quality. This alone may be responsible for the greater rate and duration of fly visits to male versus female plants, as long as reliable cues, e.g., visual or olfactory, alert them to sex differences in nectar (Atsatt and Rundel 1982; Kay et al. 1984; Ashman and Stanton 1991; Delph and

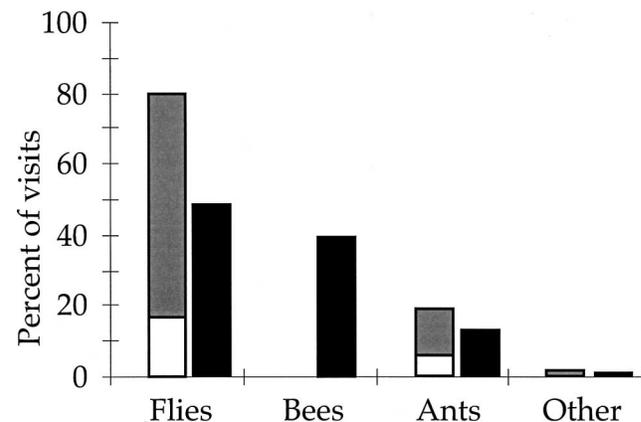


Fig. 5 Visitation by insects to flowers of female (open bars), male (gray bars), and cosexual plants (black bars) of *Wurmbea dioica* at a sympatric site near Perth, Western Australia. The percentage of visits made by flies, bees, ants, and other insects during 27.5 h of observation are shown. Percentages are calculated within sexual system ($n = 275$ visits to unisexuals, 602 to cosexuals).

Table 4

Patterns of Visitation by Flies on Plants from Monomorphic and Dimorphic Populations of *Wurmba dioica* at a Sympatric Site near Perth, Western Australia

Sex	Visit rate per flower	Duration of visit per flower (s)	Frequency of repeat visits
Female	0.7 (0.1)	69 (11)	0.57
Male	1.9 (0.4)	127 (13)	0.68
Cosexual	5.1 (0.9)	134 (15)	0.30

Note. We made observations during 15-min periods from June to August 1996 ($n = 65$ periods for males and females; $n = 45$ periods for cosexuals). Means (and SE) for the visitation rate per flower, visit duration per flower (s), and the frequency of repeat visits per plant per observation period are shown.

Lively 1992; Ashman 2000; Ashman et al. 2000). Although we found only small differences in actual flower size between males and females, apparent flower size (i.e., flower diameter) was consistently different between females and males and may act as a visual cue to floral visitors. Differences in tepal shape or area are also possible cues because nectary area has been shown to increase with tepal area in *W. dioica* ssp. *dioica* (Vaughton and Ramsey 1998). Larger flower size may also explain the greater number of flies visiting cosexuals.

The absence of strong sexual dimorphism in flower number within dimorphic populations of *W. dioica* ssp. *alba* is in striking contrast to patterns observed in gender-dimorphic *W. dioica* ssp. *dioica*. In eastern Australia, males produce significantly more flowers than do females (Barrett 1992; Vaughton and Ramsey 1998). Furthermore, flower number, but not flower size, is associated with more frequent visitation by native bees, flies, and butterflies in ssp. *dioica* (Vaughton and Ramsey 1998). In contrast to these findings, we found that flies discriminated between females and males and that flower number had no effect on visitation rates. Patterns of pollinator preference and pollinator type likely contribute to selection for sexual dimorphism in various traits (Eckhart 1991) and could therefore explain these observed differences between the subspecies of *W. dioica*.

Potential for Self-Pollination

The foraging strategies of floral visitors largely determine their effectiveness at dispersing pollen between plants. We observed small nectarivorous flies foraging unsystematically on each sex—visiting multiple flowers per plant, often making repeated visits to the same flower, and foraging for long periods of time within a small area. In contrast, individual honeybees made short visits to flowers and avoided repeat visits. Given these differences in foraging behavior, fly visitation should result in significantly more self-pollination than visitation by larger pollen-collecting bees where opportunities for selfing exist.

All three sex morphs were visited by the same suite of flies, while only cosexuals were visited by honeybees. Visitation by pollen-collecting insects may have important functional consequences for the mating system because a larger proportion of pollen is rapidly removed, making it unavailable for transfer to self-stigmas. Flies contact both female and male sex or-

gans incidentally while foraging for nectar and should have more potential to mediate self-pollination. Whether these differences translate into higher selfing rates for cosexuals versus fruiting males depends on whether opportunities for selfing are similar between the two sexes and whether flies are equally effective at pollen transfer within the sexual systems.

Regardless of visitation patterns by pollinators, selfing requires that pollen is available and that stigmas are receptive concurrently within plants. Because stigmas of *W. dioica* ssp. *alba* are receptive throughout the lifetime of ovuliferous flowers, traits controlling variation in this requirement include the degree of protogyny, the number and sex of simultaneously open flowers, and rates of pollen presentation, removal, and deposition. Differences in floral design and phenology between the sexual systems increase the likelihood of selfing for cosexuals relative to males (table 7). However, the three traits that are likely to favor selfing in cosexuals become much less influential when combined with high rates of pollen removal and deposition. Almost all of the available pollen was removed from cosexuals on the first day of pollen presentation—an average of 51.8% (± 0.03) of the anthers dehiscid and 50.3% (± 6.9) of the total pollen per flower was

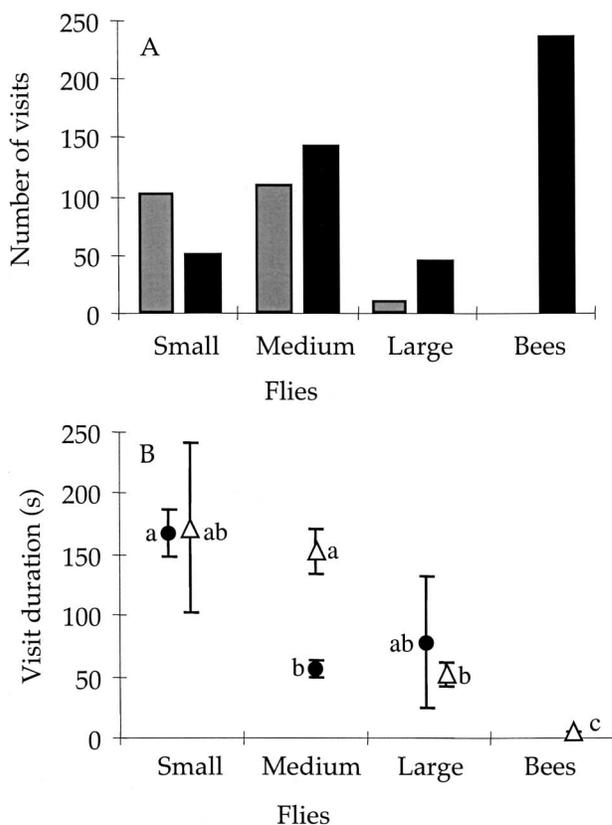


Fig. 6 Number (A) and mean (B; ± 1 SE) duration of visits by four sizes of insects to plants in monomorphic (black bars, triangles) and dimorphic populations (gray bars, circles) of *Wurmba dioica* at a sympatric site near Perth, Western Australia. Flies were classed into three size categories based on their body length; all flies are smaller than bees.

Table 5
ANCOVA of Pollen Removal and Deposition for Flowers in
Monomorphic and Dimorphic Sexual Systems of
Wurmbea dioica at a Sympatric Site
near Perth, Western Australia

Source	Proportion pollen removed	Number of pollen grains deposited
Sexual system	72.5 ^{****}	1.5
Sex (sexual system)	1.35 ^a	11.2 ^{b,***}
Days	103 ^{****}	58.6 ^{****}
Flower number	1.72	17.1 ^{****}
Flower size	7.37 [*]	2.69

Note. We assessed variation between sexual systems ($df = 1$) in the proportion pollen removed per flower and the number of pollen grains deposited per flower over a 9-d period following anther dehiscence or anthesis, respectively. We used flower size and number and the number of days of activity (all $df = 1$) as covariates. For the analysis, we logit transformed proportion pollen removed and natural log transformed total number of pollen grains deposited. *F*-values are shown; *P*-values are indicated by superscripts.

^a $df = 2$: females excluded, sex effect compares staminate and perfect flowers of cosexual and male plants.

^b $df = 1$: sex effect compares traits of female and male plants in dimorphic populations.

** $P < 0.01$.

*** $P < 0.001$.

**** $P < 0.0001$.

removed (cf. males on day 1: $38.5\% \pm 0.03\%$ of anthers dehiscid, $24.4\% \pm 6.7\%$ of pollen removed). In addition, cosexual plants received a substantial amount of pollen before any self-pollen was available—up to several hundred grains by day 3—giving precedence to outcross pollen.

The design of perfect flowers in male plants increases the likelihood of selfing both by creating opportunity and by enhancing the effectiveness of small nectar-foraging insects as pollinators. Small flower size, particularly small flower diameter, and recurved styles not only bring the sex organs closer

together but also bring them closer to the nectaries (fig. 1). In contrast, the large open flowers and erect styles of cosexuals result in a relatively larger distance between nectaries and anthers and particularly between nectaries and stigmas. Thus, nectar feeders must be larger in size to effect pollen transfer among cosexuals, and even relatively small pollen collectors are more likely to contact stigmas. Hence, small flies are likely to be much less effective pollinators of cosexuals than of unisexuals.

Relative Pollen and Seed Fitness

Differences between females and males (i.e., sexual dimorphism) may arise following the evolution of gender dimorphism in traits associated with increased reproductive success via one or the other sex function (reviewed in Delph 1996; Geber 1999). Traits enhancing male fitness may also be expected to diverge between males in dimorphic populations and cosexuals in closely related monomorphic populations. This is because even though both morphs may be phenotypically hermaphroditic, males are expected to obtain a greater proportion of their reproductive fitness through pollen than are cosexuals (Lloyd 1976). Pollen metering (i.e., limiting the amount of pollen available at any given time) enhances pollen dispersal, and hence male fitness, and it favors separate sexes by creating nonsaturating male gain curves (Charnov 1982; Charlesworth 1984; Harder and Thomson 1989; Thomson et al. 1989). There has been little empirical evidence to date of the importance of pollen metering in the context of the evolution of gender dimorphism.

Males of *W. dioica* ssp. *alba* engaged in more pollen metering than did their cosexual relatives—greater staggering of anthesis within inflorescences, more protracted anther dehiscence within flowers, and reversible dehiscence of individual anthers. Each of these mechanisms meters the amount of pollen available at any given time, which is expected to enhance male reproductive success. Because a relatively constant proportion of available pollen reaches the stigmas of other plants, pollen metering maximizes the total number of

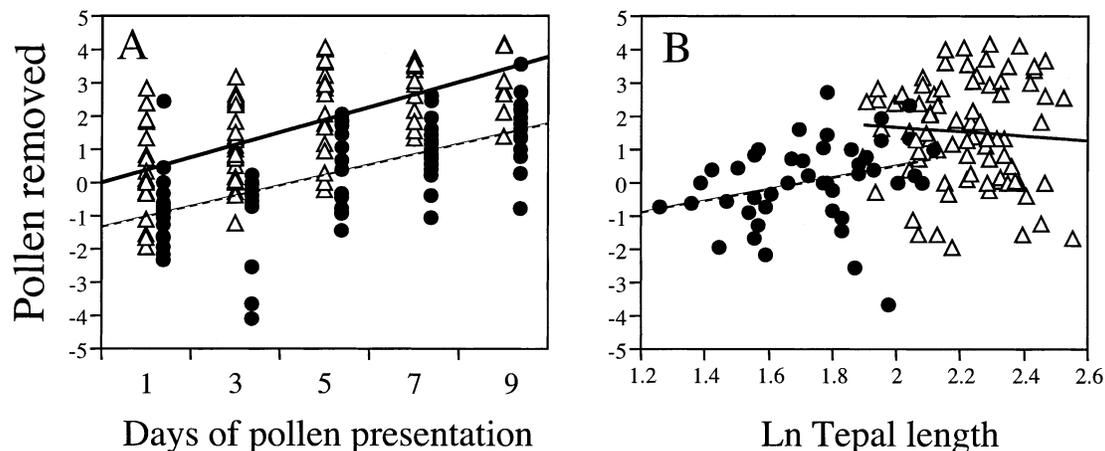


Fig. 7 Pollen removal from flowers of male (circles, dashed lines) and cosexual plants (triangles, heavy lines) of *Wurmbea dioica* at a sympatric site near Perth, Western Australia. A, Rate of pollen removal over a 9-d period of pollen presentation. B, Proportion of pollen removed relative to flower size. The ordinate represents the log-odds of the proportion of pollen removed: 1% pollen removed = -4.60 ; 99% pollen removed = 4.60 .

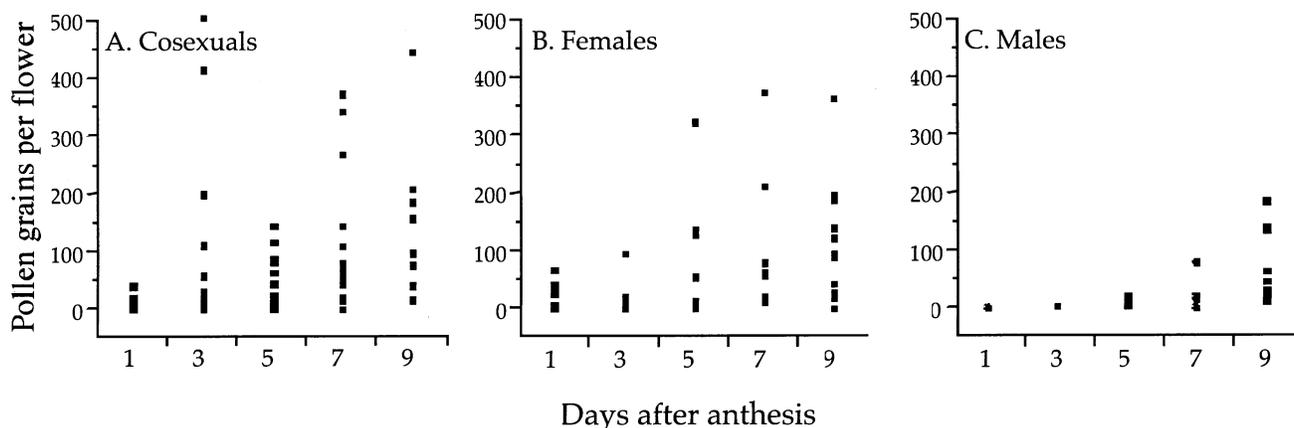


Fig. 8 Pollen deposition to ovuliferous flowers of female, male, and cosexual plants of *Wurmbea dioica* over a 9-d period following anthesis at a sympatric site near Perth, Western Australia.

successfully dispersed pollen grains (Harder and Thomson 1989). Two lines of evidence support the assertion that the observed differences in reproductive phenology in *W. dioica* ssp. *alba* enhance male reproductive success. First, both males and cosexuals exhibited staggered anthesis, while females did not, maintaining all of their flowers open for the bulk of their flowering. Second, rates of pollen deposition to cosexual and female plants were equivalent, despite the fact that the pollen pool among cosexuals was substantially larger than that for unisexuals (i.e., in monomorphic populations, pollen production per flower is three to five times greater than in dimorphic populations and is available in every flower). This indicates that in the dimorphic population, a greater proportion of the pollen of males successfully reaches stigmas.

Flower size and number are also expected to enhance reproductive success via male function (reviewed in Delph 1996; Delph et al. 1996; Eckhart 1999), although flower size has also been shown to enhance pollen deposition to females (Ashman 2000). We found no effect of flower size on either pollen removal or deposition, whereas flower number increased pollen deposition to flowers, particularly among females. Because flower number had no effect on insect visitation, greater deposition probably resulted because plants with more flowers also had greater floral longevity, allowing more time for pollen to accumulate on stigmas (Schoen and Ashman 1995).

The results from our pollinator exclusion experiment indicate that floral longevity of females and cosexuals is regulated by pollen receipt, as has been shown for many other species (Devlin and Stephenson 1984; Richardson and Stephenson 1989; Ashman and Stanton 1991; Schoen and Ashman 1995; but see Bell and Cresswell 1998). Bagging female and cosexual inflorescences significantly increased floral longevity, while the longevity of flowers on males was either unaffected (perfect flowers) or significantly shortened (staminate flowers) in the absence of visitation. This sex-specific effect of bagging, and the differential responses of fruiting versus nonfruiting males, indicates a different mechanism governing floral longevity in males. At least one study has shown that floral life span can be regulated by both pollen

receipt and pollen removal (Bell and Cresswell 1998). It is possible that our daily bagging and unbagging of polleniferous flowers dislodged some pollen from anthers, mimicking pollen removal. Thus, if the floral longevity of males is determined by pollen removal, they would have been expected to senesce sooner in the bagged treatment. The absence of an effect of bagging on perfect flowers of males suggests that both pollen removal and receipt may influence floral longevity but in opposite directions.

The female fertilities of all sexes of *W. dioica* ssp. *alba* were generally lower than might have been expected. Among angiosperm species, the average fruit set of self-compatible cosexuals is 72.5% ($n = 129$ species) and that of dioecious species is 73.8% ($n = 49$ species; Sutherland and Delph 1984). Mean fruit set of female *W. dioica* ssp. *alba* plants

Table 6

ANCOVA of the Female Fertility of Plants in Monomorphic and Dimorphic Sexual Systems of *Wurmbea dioica* at a Sympatric Site near Perth, Western Australia

Source	Ovules per flower	Fruit set	Seeds per fruit
Sexual system	30.8****	0.109	9.09*
Sex (sexual system)	1.51	3.83	3.97*
Treatment (sex, sexual system)	...	7.42	0.16
Number of ovuliferous flowers	4.48**

Note. We assessed variation between sexual systems ($df = 1$) and between females and fruiting males ($df = 1$) in the number of ovules per flower, fruit set per flower, and seed set per fruit, as well as the effect of pollination treatment (open, crossed, and selfed, $df = 5$) on fruit and seed set. We included the total number of ovuliferous flowers per plant as a covariate for ovule number per flower. We provide F -values for ovules per flower and seeds per fruit and likelihood-ratio χ^2 for fruit set. P -values are indicated by superscripts.

* $P < 0.05$.

** $P < 0.01$.

**** $P < 0.0001$.

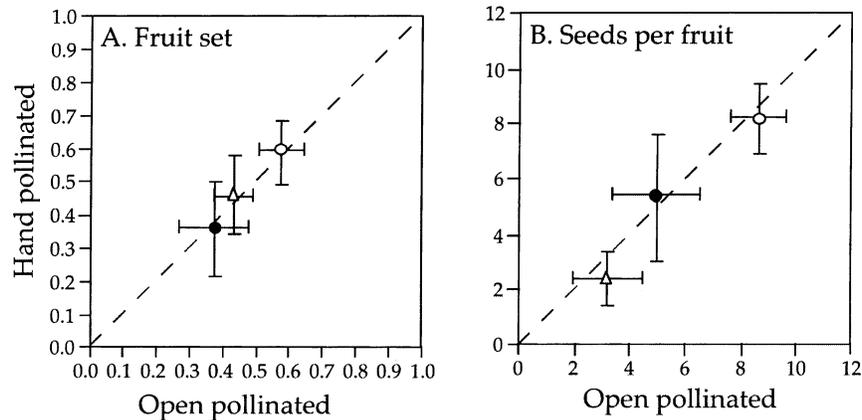


Fig. 9 Comparisons of fruit set (A) and total seeds per fruit (B) of open-pollinated and cross-pollinated flowers of female (open circles), male (closed circles), and cosexual plants (triangles) of *Wurmbea dioica* at a sympatric site near Perth, Western Australia. Means and SE are shown. Dashed lines represent equivalent fruit and seed set between pollination treatments.

was only 60%, and only 5%–7% of the ovules of cosexuals were successfully matured into seed. The cause of low seed fertility is not known, but given our results, resource limitation is a more likely cause than pollen limitation. Data from *W. dioica* in both eastern and Western Australia indicate that seed : ovule ratios are typically low, with only 25%–40% of ovules maturing into seed with no evidence of pollen limitation (Barrett 1992; Vaughton and Ramsey 1998). Vaughton and Ramsey (1998) reported an increase in the seed production of females in some populations following resource supplementation.

Table 7

Potential Effects of Reproductive Traits on the Relative Selfing Rates (s) of Cosexuals (C) and Males (M) in *Wurmbea dioica* ssp. *alba*

Trait	Effect on s	Reason
Floral sex ratio	C > M	More perfect flowers on C
No. open flowers	C > M	More open flowers on C inflorescences
Pollen production	C > M	More pollen available on C
Clonality	M > C	M multiple flowering ramets increases geitonogamy
Flower size	M > C	M flowers smaller, sex organs and nectaries closer together
Style morphology	M > C	M recurved styles put stigmas closer to anthers and nectaries
Degree of protogyny	M > C	M less time to receive outcross pollen before self-pollen is available
Pollen removal	M > C	Slower removal from M, pollen available longer
Rate of anther dehiscence	M > C	Protracted dehiscence of M, continuous source of self-pollen
Anther versatility	M > C	M anthers versatile, pollen more readily dislodged

Evolutionary Implications

Sex-specific patterns of floral design and display, the quantity and quality of reward, and reproductive phenology represent alternative strategies for pollinator attraction and the avoidance of self-pollination. We propose that differences between the sexual systems in these traits, particularly flower size, may have mediated a shift in the effectiveness of nectar-foraging insects as pollinators, leading to the evolution of sexual dimorphism from cosexuality in *W. dioica* ssp. *alba*. According to this hypothesis, sexual dimorphism originated to enforce outcrossing under pollination conditions that would have otherwise favored selfing in cosexual populations (Ganders 1978). An increase in the selfing rate in cosexual populations resulting in inbreeding depression could have provided conditions suitable for the spread of unisexual plants (Charlesworth 1999). Regardless of whether this evolutionary scenario is correct, the current differences in pollinator service, in combination with the contrasting flowering phenologies of the two sexual systems, are likely to contribute to the maintenance of combined versus separate sexes in sympatric populations of *W. dioica* ssp. *alba* in Western Australia.

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