

Maintenance of gynodioecy in *Wurmbea biglandulosa* (Colchicaceae): gender differences in seed production and progeny success

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Abstract. In gynodioecious species, females contribute genes to future generations only through ovules, and to persist in populations they must have a compensatory advantage compared with hermaphrodites that reproduce via ovules and pollen. This compensation can result from greater fecundity and/or superior success of progeny from females. We examined differences in seed production and progeny success between females and hermaphrodites in the geophyte *Wurmbea biglandulosa* to explain the maintenance of females. Females produced more ovuliferous flowers and had more ovules per flower than did hermaphrodites but this did not necessarily result in greater fecundity, in part because seed production of females was pollen-limited. Over four years in one population, open-pollinated females produced 1.32 more seeds than open-pollinated hermaphrodites (range 1.09–1.63). In two other populations examined for one year only females produced 1.07 and 0.79 as many seeds as hermaphrodites. Seed production of open-pollinated females and hermaphrodites was only 55% and 73% that of cross-pollinated plants, respectively, indicating that both genders were pollen-limited but females more so than hermaphrodites. Open-pollinated seeds from females were 1.18–1.27 times more likely to germinate than seeds from hermaphrodites. No gender differences existed in seedling growth or survival. Hermaphrodites were

self-compatible, but selfed seed set was only 80% that of crossed seed set. Crossed seed set of females and hermaphrodites did not differ. Assuming nuclear control of male sterility, relative female fitness is insufficient to maintain females at their current frequencies of 17%, and substantial female fitness advantages at later life-cycle stages are required.

Key words: Gynodioecy, inbreeding depression, male-sterility, plant breeding systems, resource compensation, sexual dimorphism, *Wurmbea biglandulosa*.

Introduction

Gynodioecy is a sexual system in which populations contain hermaphrodite and female individuals. Hermaphrodites contribute genes to subsequent generations through pollen and ovules, whereas females contribute to the next generation only via ovules. The persistence of females in populations containing hermaphrodites requires that females compensate for the absence of male function by surpassing hermaphrodites in some aspect of fitness. Possibilities include greater seed production by females or greater success of

progeny from females compared to progeny from hermaphrodites (Lewis 1941, Lloyd 1975, Charlesworth and Charlesworth 1978). The magnitude of the female fitness advantage required to maintain females in populations depends upon the mode of inheritance of male sterility. Under nuclear control, females must have a greater than twofold fitness advantage to allow a stable coexistence of both sexual morphs, whereas if nuclear and cytoplasmic genes are involved, then the female fitness advantage can be less (Lewis 1941, Lloyd 1975, Charlesworth and Charlesworth 1978, Charlesworth 1981, Frank 1989).

High relative seed production of females compared to hermaphrodites has been reported in some gynodioecious species (Shykoff 1988, Delph and Lloyd 1991, Wolfe and Shmida 1997, Ashman 1999), although in many species the female fecundity advantage is less than twofold as required by nuclear models (Kohn 1989, Eckhart 1992, Maki 1993; reviewed by Lloyd 1976, Couvet et al. 1990, Webb 1999). Moreover, in some species females and hermaphrodites have similar seed production or females produce fewer seeds (Philipp 1980, Stevens 1988, Molina Freaner and Jain 1992, Ashman 1994, Alonso and Herrera 2001). This suggests the importance of examining other life-cycle stages to determine whether progeny from females are more successful than progeny from hermaphrodites. In some gynodioecious species, seeds from females are larger or are more likely to germinate and seedlings have greater growth and survival than progeny from hermaphrodites (Assouad et al. 1978, Philipp 1980, Kohn 1988, Stevens 1988, Sakai et al. 1997, Wolfe and Shmida 1997).

A female fitness advantage in seed production or progeny success may arise from several possible causes. Resource compensation hypotheses assume that because females do not produce pollen and often have lower expenditure on pollinator attraction, they have more available resources for seed production than hermaphrodites (Lewis 1941). Such hypotheses assume that resources rather than pollen limit

seed set of females. If pollinators are scarce, however, then females may produce fewer seeds than hermaphrodites if the latter are self-compatible (Fleming et al. 1998). Inbreeding avoidance hypotheses assume that seeds from females are obligately outcrossed, whereas seeds from self-compatible hermaphrodites may be the products of self-fertilization. Progeny from hermaphrodites may suffer inbreeding depression and exhibit reduced fitness, due to increased homozygosity and the expression of deleterious recessive alleles (Lloyd 1975; Charlesworth and Charlesworth 1978, 1987). Most studies of gynodioecious species have indicated a role for inbreeding avoidance, and in some cases resource compensation, in the maintenance of females in populations (Kohn 1988, 1989; Shykoff 1988; Ashman 1992, 1999; Eckhart 1992; Kohn and Biardi 1995; Schultz and Ganders 1996; Sakai et al. 1997; Thompson and Tarayre 2000).

The objective of this study was to quantify fitness differences between females and hermaphrodites necessary for the maintenance of gynodioecy in *Wurmbea biglandulosa*. Specifically, we (1) document female frequency in populations, (2) determine differences in open-pollinated seed production between females and hermaphrodites, (3) assess differences in the quality of progeny from open-pollinated females and hermaphrodites, (4) determine self-compatibility of hermaphrodites and whether natural seed set of both sexual morphs is pollen-limited, and (5) examine gender differences in vegetative and reproductive traits that may be related to resource-compensation effects.

Materials and methods

Study species and sites. *Wurmbea biglandulosa* (R. Br.) T.D. Macfarl. spp. *biglandulosa* (Colchicaceae) is a perennial geophyte that occurs in a variety of habitats in eastern Australia from southeastern Queensland to northeastern Victoria. Flowering plants have a corm, a single stem, 2–3 annual leaves, and an inflorescence spike with 1–6 flowers. Flowers have six white tepals (8–10 mm long), each with two nectaries located towards the

base (Macfarlane 1980). Flowering occurs in spring (August–October) and generalist insect pollinators, particularly flies, visit flowers for nectar. Populations are either hermaphroditic or gynodioecious with up to 44% females (Vaughton and Ramsey 2002). Hermaphroditic plants are sex labile and comprise several forms; plants that produce all perfect flowers, plants with lower perfect flowers and terminal polliniferous only flower(s) and plants with all polliniferous only flowers (i.e. males). The latter non-fruiting hermaphrodites occur in low frequencies in all populations and are uniformly small in size (Vaughton and Ramsey 2002). Females produce ovuliferous only flowers. Most of these flowers have neither filaments nor anthers, although occasional flowers have shortened filaments, and rare flowers (<5%) have shortened filaments and shrivelled anthers with no pollen (Vaughton and Ramsey 2002).

We conducted this study in three gynodioecious populations (Geehi, Grassy Flat, Tom Groggin; populations 29–31 in Vaughton and Ramsey 2002) in Kosciuszko National Park, in the Snowy Mountains region of southern New South Wales (36°33'S; 148°09'E). Populations were located in disturbed open forest and were ca. 15 km apart.

Female frequencies. To assess variation in female frequencies among years and populations we scored sex ratios during peak flowering in 1995–1998 (total N=5004 plants). The numbers of female, hermaphrodite and male phenotypes were scored as described in Vaughton and Ramsey (2002). The sex of at least 1000 plants or all plants was scored while walking transects through populations. Hermaphrodite and male phenotypes were pooled and we used a multinomial logit model and analysis of deviance assuming a Poisson error distribution to examine the effects of population and year on female frequencies. The mean residual deviance was significant ($\chi^2 = 31.39$, $df = 6$, $P < 0.001$), and we subsequently used the more conservative deviance ratio, which approximates the F-distribution (Collett 1991) to examine the significance of population and year.

Seed production. We examined ovule and open-pollinated seed production of females and hermaphrodites in the Grassy Flat population from 1995–1998, and the Geehi and Tom Groggin populations in 1998. In *W. biglandulosa*, sex lability of polliniferous phenotypes may influence the maintenance of gynodioecy if hermaphrodites lose

opportunities to produce seeds in years when they flower as males. To account for this possibility we included in our sample hermaphrodite and male phenotypes that were selected in proportion to their frequencies in the total pool of polliniferous plants (ca. 88% and 12%, respectively). Plants (N=20 of each sex) were selected haphazardly as flowers opened and fruits were harvested 6 weeks later. The number of seeds and unfertilised ovules in the first fruit and the number of seeds in all fruits were counted using a dissecting microscope. To compare the conversion of ovules to seeds independently of the number of ovules per flower, we calculated the percentage of ovules that produced seeds in the first flower. For ovule production, percent seed set and seed production per plant we assessed the effects of sex and year (or population) with two-way analyses of variance (ANOVA).

To compare seed mass of the sexual morphs we weighed 15 seeds individually from each of 15 plants of each sex in each population in 1998. Seeds were collected from the field as fruit capsules were opening, and were stored for 6 months in the laboratory before weighing. We assessed the effects of sex and population, and plants nested within populations on seed mass using a partially nested ANOVA.

Seed germination and seedling growth. To compare germination of seeds produced by females and hermaphrodites we used seeds collected in 1998 from the Grassy Flat and Tom Groggin populations. From each population, 30 seeds from each of 15 maternal plants of each sex were surface-sterilized with dilute bleach solution and placed on moistened filter paper in glass Petri dishes (30 seeds dish⁻¹). Seeds were chilled at 5 °C for 30 days to break seed dormancy and dishes were randomly arranged in a growth cabinet at 16 °C:8 °C for a 12 h light:12 h dark photoperiod. Dishes were kept moist and relocated each fortnight to avoid position effects. Many seeds from Grassy Flat germinated within 4 months (>56%) but seeds from Tom Groggin were slow to germinate (<20%). To stimulate further germination all seeds from both populations were placed at 5 °C for 14 days after 4 months and again after 10 months; on each occasion there was an increase in germination when seeds were returned to higher temperatures. Final germination percentages for each plant in both populations were calculated after 14 months. We assessed the effects of sex and population on

percent seed germination using a two-way ANOVA with maternal plants as replicates.

For the Grassy Flat population we assessed differences in emergence, growth and survival of seedlings from female and hermaphrodite plants in a phytotron. We used different maternal plants from those in the germination experiment. For each sex, 14–28 seeds from each of 18 maternal plants (females: $n = 368$; hermaphrodites: $n = 478$), were sown to a depth of 5 mm in 48-cell seedling trays (one seed cell⁻¹; cell volume 90 cm³), containing soil comprised of equal parts loam, sand and peat. Trays were chilled at 5 °C for 30 days to break seed dormancy and then placed in a phytotron set at 16 °C:8 °C for a 12 h light: 12 h dark photoperiod. Trays were kept moist and relocated each fortnight to avoid position effects.

Seedling emergence was monitored for 6 months; most seedlings emerged before 4 months. We monitored seedlings for the next 18 months. All seedlings grew for about 6–7 months before leaves senesced and plants became dormant. Plants remained in a dormant state for 3–5 months before resprouting new leaves from the underground corm. We assessed seedling size at the end of the first year by measuring cumulative length of leaves which is related to plant dry weight (dry weight = $1.81 + 0.91$ length of leaves, $F_{1,38} = 370.76$, $P < 0.001$ $r^2 = 0.91$). We examined plant survival in the second year by scoring the number of plants that emerged from dormancy. Any plants that failed to emerge were excavated; in all cases corms were absent, indicating that plants were dead. Gender differences in percent seedling emergence and survival in the phytotron were examined with one-way ANOVAs with maternal plants as replicates. For seedling size, we examined differences between the sexual morphs using a nested ANOVA with maternal plants nested within sex, and seedlings as replicates.

Hand pollination experiment. We conducted a pollination experiment in the Grassy Flat population in 1997 to determine whether (1) hermaphrodites were self-compatible and (2) seed set of open-pollinated plants was pollen-limited. We cross-pollinated females, cross- and self-pollinated hermaphrodites, and marked nearby open-pollinated females and hermaphrodites as controls. Only hermaphrodites with at least one perfect flower were used. Manually pollinated plants were bagged with fine mesh to exclude insect visitors. All

ovuliferous flowers on plants were pollinated on the day of opening and on two subsequent occasions to ensure that pollen deposition was not limiting. Selfed flowers received fresh pollen from the same flower or another newly opened flower on the same plant. Crossed flowers were emasculated and pollinated by at least two different donors at least 5 m distant. Open-pollinated plants were left untouched. Fruits were harvested after 6 weeks and the number of seeds produced by plants was counted. We compared seed production of the following treatments with one-way ANOVAs to address the questions posed above: (1) self- vs. cross-pollinated hermaphrodites, and (2) cross- vs. open-pollinated plants, followed by *a posteriori* Student-Newman-Keuls tests.

Sexual dimorphism. We assessed sexual dimorphism at Grassy Flat and Tom Groggin by determining the total number of flowers, the number of ovuliferous flowers, cumulative length of the first two leaves, stem height and flower diameter on 50 female and hermaphrodite plants. We included in our sample hermaphrodite and male plants for reasons described previously. Measurements were undertaken as described in Vaughton and Ramsey (2002). We examined the effects of sex and population on traits with two-way ANOVAs.

For a subset of 20 plants of each sex at Grassy Flat, we estimated resource allocation to flowers by determining the dry mass of the first flower. Tepals, gynoecium and androecium, the latter for hermaphrodites only, were separated, dried to constant weight at 60 °C and weighed to the nearest 0.1 mg. Stamens had undehiscent anthers and thus dry mass of pollen was included. We used one-way ANOVAs to test for gender differences in allocation.

Statistical analyses. For ANOVAs, measurements and the numbers of flowers (+ 1), ovules and seeds were transformed using natural logarithms. Percentages were square-root arcsine transformed. Transformation improved normality and homoscedasticity as established by Shapiro-Wilk normality and Levene's tests, respectively. All two-way ANOVAs were Model III, with sex as a fixed factor, and year or population as random factors. Nonsignificant interactions ($P > 0.14$) were omitted in final analyses to increase degrees of freedom for testing gender differences (Sokal and Rohlf 1995). Means (\pm SE) are given.

Results

Sex ratios. The frequency of females was not dependent on either year or population (year: $F_{3,6} = 1.40$, $P = 0.33$; population: $F_{2,6} = 0.39$, $P = 0.71$). Pooled over years and populations, female frequency was $17.0 \pm 1.5\%$. Pooled frequencies for hermaphrodites and males were $72.2 \pm 1.8\%$ and $10.8 \pm 1.4\%$, respectively.

Seed production. At Grassy Flat, there were significant differences between females and hermaphrodites in the numbers of ovules per flower and seeds per plant (Table 1). Averaged over the four years, females produced 1.08 times as many ovules (range, 0.99–1.17) and 1.32 times as many seeds as did hermaphrodites (range, 1.09–1.63). There was also a trend for females to have higher percent seed set, although the gender difference did not reach statistical significance. No significant variation occurred among years for any of the traits (Table 1).

For the three populations in 1998, the sexes differed in ovule production (Table 2). Females produced on average 1.18 times as many ovules per flower as did hermaphrodites. For percent seed set, the gender \times population interaction was significant; females had significantly higher seed set at Grassy Flat, whereas hermaphrodites had significantly higher seed set at Tom Groggin. At Geehi, seed set did not

differ between the sexual morphs (Table 2). For the number of seeds per plant there was a trend for females to produce more seeds which was largely due to sex differences in the Grassy Flat population (female:hermaphrodite ratio 1.63). At Geehi and Tom Groggin females produced 1.07 and 0.79 times as many seeds as hermaphrodites, respectively. Variation in seed production among populations was significant (Table 2).

Seed mass differed significantly between the sexual morphs ($F_{1,1304} = 65.01$, $P < 0.001$) but not among populations ($F_{2,42} = 0.56$, $P = 0.576$). Seeds from females weighed 10% less than seeds from hermaphrodites (1.8 ± 0.02 mg vs. 2.0 ± 0.02 mg). Variation in seed mass among plants within populations was significant ($F_{42,1304} = 11.15$, $P < 0.001$).

Seed germination and seedling growth. Gender had a significant effect on seed germination ($F_{1,57} = 5.78$, $P = 0.019$). Seeds from females were 1.18 and 1.27 times more likely to germinate than seeds from hermaphrodites at Grassy Flat and Tom Groggin, respectively (Grassy Flat: $94.5 \pm 1.3\%$ vs. $80.4 \pm 7.4\%$; Tom Groggin: $59.5 \pm 5.4\%$ vs. $46.7 \pm 6.4\%$). More seeds germinated at Grassy Flat than at Tom Groggin ($F_{1,57} = 43.85$, $P < 0.001$).

In the phytotron, seedling emergence was 13% greater for seeds from females than

Table 1. Comparison of ovule and seed production of females and hermaphrodites at Grassy Flat from 1995–1998. Means (\pm SE) and F-ratios for ANOVAs are presented. The sex \times year interactions (all $P > 0.44$) were omitted from the final analyses. Degrees of freedom are in parentheses

Year	Sex	Number of ovules/flower	Percent seed set	Number of seeds/plant
1995	Female	73.7 ± 3.8	23.6 ± 3.2	41.6 ± 6.3
	Hermaphrodite	64.9 ± 6.5	18.7 ± 3.3	30.4 ± 5.8
1996	Female	73.7 ± 3.1	23.4 ± 3.5	45.9 ± 7.8
	Hermaphrodite	74.6 ± 6.3	24.7 ± 3.8	42.2 ± 6.3
1997	Female	65.5 ± 2.0	21.7 ± 2.6	45.3 ± 7.1
	Hermaphrodite	65.9 ± 5.9	22.8 ± 3.7	38.7 ± 6.6
1998	Female	83.8 ± 3.9	30.4 ± 3.6	56.2 ± 4.6
	Hermaphrodite	71.6 ± 6.3	20.5 ± 2.7	34.4 ± 3.6
F-ratio	Sex (1,155)	6.91^{**}	2.73^{\dagger}	7.34^{**}
	Year (3,155)	0.21^{ns}	0.57^{ns}	0.89^{ns}

^{ns} $P > 0.05$, [†] $P = 0.10$, ^{**} $P < 0.01$

Table 2. Comparison of ovule and seed production of females and hermaphrodites at Grassy Flat, Geehi and Tom Groggin in 1998. Means (\pm SE) and F-ratios from ANOVAs are presented. For numbers of ovules and seeds, the sex \times population interactions (both $P > 0.14$) were omitted from the final analyses. For seeds per plant, means within populations with different superscripts differed significantly (Student-Newman-Keuls tests, $P < 0.05$). For percent seed set, the interaction was significant and means within populations with different superscripts differed significantly ($P < 0.05$), following tests of the simple main effect of gender (Underwood 1997). Degrees of freedom are in parentheses

Population	Sex	Number of ovules/flower	Percent seed set	Number of seeds/plant
Grassy Flat	Female	83.8 \pm 3.9	30.4 \pm 3.6 ^a	56.2 \pm 4.6 ^a
	Hermaphrodite	71.4 \pm 6.3	19.8 \pm 2.7 ^b	34.4 \pm 3.7 ^b
Geehi	Female	86.7 \pm 3.2	33.3 \pm 2.8 ^a	62.9 \pm 8.9 ^a
	Hermaphrodite	78.2 \pm 6.6	32.8 \pm 3.5 ^a	58.6 \pm 6.6 ^a
Tom Groggin	Female	87.8 \pm 3.5	12.7 \pm 2.2 ^b	27.6 \pm 4.9 ^a
	Hermaphrodite	70.3 \pm 6.3	24.6 \pm 2.7 ^a	35.0 \pm 4.2 ^a
F-ratio	Sex (1,116)	7.95 ^{**}	–	2.68 [†]
	Population (2,116)	0.05 ^{ns}	–	4.73 [*]
	Sex \times pop (2,114)	–	5.67 ^{**}	–

^{ns} $P > 0.05$, [†] $P = 0.10$, ^{*} $P < 0.05$, ^{**} $P < 0.01$

from hermaphrodites, although the difference was not significant ($73.5 \pm 4.5\%$ vs. $65.1 \pm 4.5\%$; $F_{1,34} = 2.54$, $P = 0.120$). Seedling size did not differ among progeny from females and hermaphrodites (94.8 ± 1.2 mm vs. 100.2 ± 1.1 mm; $F_{1,34} = 2.11$, $P = 0.155$), although variation among plants within sexes was significant ($F_{34,540} = 3.27$, $P < 0.001$). A similar proportion of progeny from females and hermaphrodites survived to their second year ($81.9 \pm 2.9\%$ vs. $85.0 \pm 2.8\%$; $F_{1,34} = 0.82$, $P = 0.371$).

Hand pollination. All hand- and open-pollinated flowers produced seeds. Hermaphrodites were self-compatible, although seed production of selfed plants was only 80% that of crossed plants (seeds plant⁻¹: 45.4 ± 4.2 vs. 57.2 ± 4.1 ; $F_{1,38} = 4.90$, $P = 0.033$). Cross-pollinated females and hermaphrodites produced significantly more seeds than open-pollinated plants, indicating that natural seed set was pollen-limited (Fig. 1). Seed production of open-pollinated females and hermaphrodites was only 55% and 73% that of cross-pollinated plants, respectively, indicating that females were more pollen-limited than hermaphrodites. Seed production of cross-

pollinated females and hermaphrodites did not differ significantly (Fig. 1).

Sexual dimorphism. Females produced 32–35% more ovuliferous flowers than did hermaphrodites, but both produced similar numbers of total flowers (Table 3). Hermaphrodites had stems that were 8–10% taller than females. Length of leaves was similar for both genders. Plants at Grassy Flat were taller and had longer leaves than those at Tom Groggin (Table 3).

Hermaphrodites produced flowers that were 17–20% larger in diameter (Table 3) and tepals that were 88% heavier than those of females (dry mass: 7.7 ± 0.5 mg vs. 4.1 ± 0.4 mg; $F_{1,38} = 30.22$, $P < 0.001$). Gynoecium mass of hermaphrodites and females did not differ (2.3 ± 0.2 mg vs. 3.1 ± 0.4 mg; $F_{1,38} = 2.06$, $P = 0.159$). Total floral mass was 67% greater for hermaphrodites than females (12.0 ± 0.6 mg vs. 7.2 ± 0.7 mg; $F_{1,38} = 30.91$, $P < 0.001$).

Discussion

Populations of *W. biglandulosa* in the Snowy Mountains area of south-eastern Australia contained on average 17% females. Females

frequency did not vary among years or populations and fell within the range reported for other *W. biglandulosa* populations (0–44%, Vaughton and Ramsey 2002). Key parameters in models explaining the maintenance of females in populations of hermaphrodites involve a female advantage in seed production and progeny success. We found only limited evidence of a female fecundity advantage, in part because seed production of females was

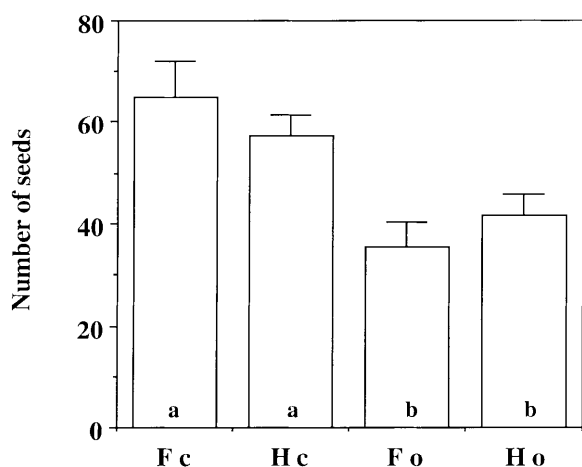


Fig. 1. Mean (\pm SE) number of seeds produced per plant at Grassy Flat for cross- and open-pollinated females and hermaphrodites (Fc, Hc, Fo, Ho). Variation among the pollination treatments was significant ($F_{3,76} = 8.35$, $P < 0.001$) and means with different letters differed significantly ($P < 0.05$, Student-Newman-Keuls tests)

pollen-limited. Seeds from females were more likely to germinate than seeds from hermaphrodites but no gender differences existed in seedling growth and survival. Below we discuss these results and their implications for the evolution of gynodioecy in *W. biglandulosa*.

Females had a fecundity advantage in the Grassy Flat population and over four years they produced on average 1.32 times more seeds than open-pollinated hermaphrodites (range 1.09–1.63). In the Tom Groggin population, however, females had a fecundity disadvantage and produced only 0.79 times as many seeds as hermaphrodites. Seed production was examined for only one year at Tom Groggin, and it is unknown whether the low relative fecundity of females reported here reflects gender differences in lifetime fecundity. At Geehi where seed production was also examined for one year both sexual morphs had similar fecundity (1.07). In other gynodioecious species, a female fecundity advantage of greater than two, consistent with nuclear models of sex determination, has been reported in some studies (e.g. half of the species reviewed in Couvet et al. 1990, also Delph and Lloyd 1991, Wolfe and Shmida 1997, Ashman 1999). However, many studies have found a female fecundity advantage of less than two or that hermaphrodites produce more seeds than females (e.g. Philipp 1980, Stevens 1988, Kohn 1989, Couvet et al. 1990, Eckhart 1992,

Table 3. Sexual dimorphism of females and hermaphrodites at Grassy Flat and Tom Groggin. Means (\pm SE) and F-ratios for ANOVAs (all $df = 1, 197$) are presented. The sex \times population interactions (all $P > 0.38$) were omitted from the final analyses

Population	Sex	Total number of flowers	Number of ovuliferous flowers	Stem height (mm)	Length of leaves (mm)	Flower diameter (mm)
Grassy Flat	Female	2.5 \pm 0.1	2.5 \pm 0.1	194.7 \pm 4.9	468.9 \pm 14.3	22.6 \pm 0.4
	Hermaphrodite	2.5 \pm 0.1	1.9 \pm 0.1	205.1 \pm 6.7	448.4 \pm 13.3	27.2 \pm 0.5
Tom Groggin	Female	2.7 \pm 0.1	2.7 \pm 0.1	157.9 \pm 7.0	394.1 \pm 11.6	22.8 \pm 0.4
	Hermaphrodite	2.6 \pm 0.1	2.0 \pm 0.1	170.9 \pm 6.7	400.9 \pm 13.6	26.6 \pm 0.4
F-ratio	Sex	0.13 ^{ns}	22.72 ^{***}	3.22 [†]	0.32 ^{ns}	106.62 ^{***}
	Population	2.55 ^{ns}	1.57 ^{ns}	34.23 ^{***}	21.01 ^{***}	0.12 ^{ns}

^{ns} $P > 0.05$, [†] $P < 0.10$, ^{***} $P < 0.001$

Molina-Freaner and Jain 1992, Maki 1993, Thompson and Tarayre 2000, Alonso and Herrera 2001), which may indicate that female advantages exist at later life-cycle stages or that sex is determined by both nuclear and cytoplasmic factors.

Our estimates of hermaphrodite fecundity were based on plants that produced perfect flowers and plants that produced polliniferous only flowers (i.e. males) in proportion to their frequencies in populations. This accounts for the fact that hermaphrodites lose opportunities to produce seeds in years when they flower as males. If only plants with perfect flowers had been used then our estimates of hermaphrodite seed production would have been greater and the magnitude of female fecundity advantage at Grassy Flat would have been reduced. At Grassy Flat, females produced more ovuliferous flowers, had more ovules per flower and converted more ovules into seeds. Greater production of ovuliferous flowers and ovules per flower by females has also been found in other *W. biglandulosa* populations (Vaughton and Ramsey 2002). In gynodioecious populations, hermaphrodites achieve much of their fitness through pollen and a female advantage in fecundity-related components could indicate selection for reduced allocation to female reproduction in hermaphrodites (Lloyd 1975, Charlesworth and Charlesworth 1978). In *W. biglandulosa*, however, allocation to female reproduction in hermaphrodites is probably more a consequence of plant size and/or resource status. Male phenotypes are consistently small and are probably either lacking resources or flowering for the first time. Also, hermaphrodite phenotypes often have polliniferous only flowers in distal positions on inflorescences (Vaughton and Ramsey 2002) and the production of perfect flowers is reduced when plants are stressed (G. Vaughton and M. Ramsey unpublished data). Collectively, these results indicate that allocation to female function in hermaphrodites is limited by the availability of resources and that size thresholds probably exist for the expression of female function. Similar effects have been

reported in subdioecious *W. dioica* (Barrett et al. 1999, Ramsey and Vaughton 2001) and in gynodioecious *Hebe subalpina* (Delph and Lloyd 1991), *Ochradenus baccatus* (Wolfe and Shmida 1997) and *Fragaria virginiana* (Ashman 1999).

Low relative seed production of females in the Tom Groggin population was probably related to pollen limitation. Although females produced more ovules per flower than hermaphrodites, they converted a lower proportion of ovules into seeds, as would be the case if fewer pollen grains were deposited on their stigmas. The hand pollination experiment conducted at Grassy Flat showed that fecundity of both sexual morphs was pollen-limited under natural conditions, but that females were more affected than hermaphrodites. Pollen limitation has been reported as a factor affecting the relative seed fecundity of females and hermaphrodites in other studies (Fleming et al. 1998). Because females rely entirely on the transfer of pollen among plants for reproduction, they are more susceptible to pollen limitation than hermaphrodites that are self-compatible. In *W. biglandulosa*, the reduced size of female flowers may have exacerbated pollen limitation because smaller flowers are often less attractive to generalist pollinators than larger flowers (Delph et al. 1996, Vaughton and Ramsey 1998, Ashman 2000). Another factor that can affect the relative seed fecundity of the sexual morphs is population structure (McCauley and Brock 1998, Graff 1999). Females are expected to suffer increasing pollen limitation, as their density or frequency increases in populations. In the present study females may have suffered greater pollen limitation than hermaphrodites if their distributions within populations tended to be more clumped. Pollen limitation may not necessarily be a common feature of other *W. biglandulosa* populations. The density of flowering plants was low in all the present study populations and weather conditions during spring flowering in the mountain habitat are frequently unsuitable for pollinators. Other populations of *W. biglandulosa* that we have observed have

much higher densities of flowering plants and a more benign climate, indicating that pollen limitation may be less important.

As in other *W. biglandulosa* populations (Vaughton and Ramsey 2002), females had smaller flowers and shorter stems than did hermaphrodites, potentially providing a basis for resource compensation. Resource compensation hypotheses assume that because females do not produce pollen and allocate fewer resources to pollinator attraction, they should have more resources available for seed production (Eckhart 1992, Ashman 1994). Females in populations studied here had an advantage in some fecundity-related components, such as production of ovuliferous flowers and ovules per flower. However, gender differences in seed production under natural conditions was minimal, in part because seed set of females was pollen-limited. Even when plants were manually cross-pollinated the difference in seed production between the sexual morphs was not statistically significant. This indicates that if resource compensation occurs in *W. biglandulosa*, then resources may be used to provide a female advantage at later life-cycle stages (e.g. Van Damme and Van Delden 1984, Stevens and Van Damme 1988).

We found that hermaphrodites were self-compatible but that selfed plants produced only 80% as many seeds as did crossed plants. Lower seed production following selfing may be related to reduced embryo survival during seed development (i.e. early-acting inbreeding depression, Charlesworth and Charlesworth 1987). The absence of significant differences in seed production of cross-pollinated females and hermaphrodites indicates that both genders are inherently similar in their potential fecundity after the deleterious effects of selfing are experimentally removed. In self-compatible species, females may produce higher quality offspring than do hermaphrodites because their unisexual condition prevents inbreeding. In *W. biglandulosa*, seeds from females were 1.17–1.28 times more likely to germinate than seeds from hermaphrodites. We found no

gender differences in seedling size or survival, although it is possible that superior success of female progeny may be apparent in the field where conditions are more stressful than in the glasshouse (e.g. Schemske 1983). Our experimental design does not allow us to speculate on whether lower germination of seeds from hermaphrodites was due to inbreeding depression or maternal effects (e.g. Ashman 1992). However, it seems likely that hermaphrodites produce some selfed seeds under natural conditions, because unspecialized insect pollinators visit flowers and plants have flowers open concurrently. In subdioecious *W. dioica*, which has similar floral morphology and pollinators, open-pollinated hermaphrodites experience moderate levels of selfing and high inbreeding depression ($s \approx 0.60$, $\delta = 0.85$; M. Ramsey and G. Vaughton unpublished). High levels of self-fertilization and inbreeding depression have also been reported in other gynodioecious species (e.g. Kohn and Biardi 1995, Schultz and Ganders 1996, Sakai et al. 1997).

Our findings for *W. biglandulosa* support the proposal by Barrett (1992) that dioecy in the genus *Wurmbea* evolved via the gynodioecious route. The three *W. biglandulosa* populations studied here have not evolved far along this pathway from gynodioecy to dioecy (Lloyd 1976, Delph and Lloyd 1991, Webb 1999). The relatively low frequency of females ($\approx 17\%$) and substantial seed production by hermaphrodites indicate that the study populations are near the hermaphrodite end of the hermaphrodite-gynodioecy-dioecy continuum. This contrasts with populations of subdioecious *W. dioica* in which female frequencies approach 50% and hermaphrodites have low seed production relative to females (Barrett et al. 1999, Ramsey and Vaughton 2001).

In gynodioecious populations female frequencies are influenced by the relative seed fitnesses of hermaphrodites and females and the genetic control of sex (Lewis 1941, Lloyd 1976, Webb 1999). Lloyd (1976) showed that when nuclear genes govern sex determination, the expected frequency of females should be:

$$C = 1 - 2p/2(1 - p),$$

where C is the relative seed fitness of hermaphrodites and p is the frequency of females in the population. This model indicates a negative relationship between C and p , such that as C decreases to zero, p increases to the maximum of 0.5. Although the mode of genetic control of male sterility in *W. biglandulosa* is unknown, we use the above model to assess the similarity between the observed relative hermaphrodite fitness (C) and the predicted relative hermaphrodite fitness (C_p), the latter being estimated from the observed female frequency.

For relative hermaphrodite seed fitness, we used the Grassy Flat data and calculated cumulative seed fitness as the product of seeds per plant (average of 4 years, hermaphrodite/female = 0.773), seed germination (0.851) and seedling survival (1.034). Substituting cumulative fitness and $p=0.17$ in the model, C exceeded substantially the value of C_p (i.e. $0.682 > 0.398$). Thus the observed female frequency is greater than expected based on our estimates of relative hermaphrodite fitness, indicating that females require a substantial fitness advantage later in their life-cycle to maintain their current frequency of about 17%. Several factors could increase female fitness relative to hermaphrodites. For example, inbreeding depression may adversely affect hermaphrodites at later life-cycle stages (Charlesworth and Charlesworth 1987), or gender differences may exist in life-history traits such as age of first reproduction (Philipp 1980), adult survival (Van Damme and Van Delden 1984) or ability to clone (Stevens and Van Damme 1988). However, if the genetic control of male sterility is nuclear-cytoplasmic and not nuclear as assumed by the model, then the observed fitness differences should be sufficient to maintain the observed female frequencies (Charlesworth 1981).

In conclusion, existing models based on nuclear sex determination cannot explain the presence of females in populations of *W. biglandulosa* given our estimates of relative

fecundity and progeny success. Further studies are required to quantify fitness differences of the sexual morphs at other life-cycle stages and to investigate the level of selfing and inbreeding depression in hermaphrodites to explain more fully the maintenance of females in these populations.

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