

Self-compatibility and floral biology in subdioecious *Wurmbea dioica* (Colchicaceae)

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Abstract. Dioecy has long been interpreted as a mechanism to avoid self-fertilisation and the negative effects of inbreeding depression. We determined relative self-compatibility and temporal overlap of male and female functions for hermaphrodites in subdioecious *Wurmbea dioica* to assess the role of selfing in the maintenance of gender dimorphism. Plants were self-compatible but did not self-pollinate autonomously. Self- and open-pollinated hermaphrodites had high seed abortion and low seed set, whereas crossed hermaphrodites and crossed and open females had low seed abortion and high seed set. For selfed hermaphrodites, abortion occurred over a range of developmental stages and was negatively related to seed set. Inbreeding depression for seed set varied extensively among plants, averaging 0.56. Despite high inbreeding depression, substantial selfing occurred in open-pollinated hermaphrodites ($S = 0.76$), as estimated by a non-genetic method. Flowers were protogynous, although temporal separation of male and female functions was limited because anthers began to dehisce shortly after flowers opened. Anther dehiscence was staggered over 4 days and floral longevity, pollen longevity and stigma receptivity were prolonged, thus providing considerable opportunities for pollinator-mediated self-pollination. Our findings indicate that high levels of selfing and subsequent abortion of selfed embryos due to early acting inbreeding depression are important factors maintaining gender dimorphism in *W. dioica*.

Introduction

Plant sexual systems are diverse and include not only hermaphroditic plants with combined male and female sexual functions, but also gender dimorphism involving the separation of the sexes in different plants (Barrett 1998). Dioecy, the coexistence of male and female plants in populations, has evolved repeatedly in many plant families (Webb 1999). The evolution of dioecy from self-compatible hermaphroditic ancestors has long been interpreted as a mechanism to promote outcrossing and to avoid self-fertilisation and the ensuing negative consequences of inbreeding depression (Darwin 1877; Lloyd 1976, 1982; Charlesworth and Charlesworth 1978; Bawa 1980; Thomson and Barrett 1981; Sakai and Weller 1999). Inbreeding depression following selfing lowers the ovule success of self-compatible hermaphrodites compared with females that produce seeds only by outcrossing. Theoretical models suggest that females can establish in hermaphroditic populations when there is a combination of high inbreeding depression and high selfing rates (Charlesworth and

Charlesworth 1978). Recent empirical studies have demonstrated that such conditions are not unrealistic and support the view that inbreeding avoidance has been an important selective factor in the evolution of separate sexes in some species (Maki 1993; Kohn and Biardi 1995; Schultz and Ganders 1996; Sakai *et al.* 1997). Gender dimorphism is less likely to evolve in self-incompatible species because females do not have an outcrossing advantage. Females thus require the necessary seed fecundity advantage, the magnitude of which depends on the genetic control of male sterility, to arise from the sole effects of resource reallocation (Charlesworth and Charlesworth 1978).

Arguments for the role of inbreeding avoidance in the evolution of dioecy have been linked to pollination biology. Inbreeding depression has been proposed to be a strong impetus for gender dimorphism in situations where a shift in pollination mode, often associated with a change in habitat, leads to an increased level of selfing (Webb 1979; Delph 1990; Barrett 1992; Weller *et al.* 1995). Because genetic load is likely to be substantial in populations that are historically

outcrossing, inbreeding depression can be severe when selfing levels are increased (Husband and Schemske 1996). Negative effects of selfing are most likely in plants that lack strong spatial or temporal separation of male and female functions within flowers. In such species, increased visits by generalist pollinators with restricted patterns of foraging can result in substantial self-pollination and subsequent inbreeding depression (Bawa 1980; Lloyd 1982).

Wurmbea dioica (R.Br.) F.Muell. is a small lilioid geophyte that is widely distributed across southern Australia (Macfarlane 1980). Populations exhibit marked intraspecific variation in sexual systems ranging from hermaphroditism, through gynodioecy and subdioecy to full dioecy (Barrett 1992; Case and Barrett 2001; Ramsey and Vaughton 2001). In Western Australia, sympatric hermaphroditic and dioecious populations occupy wet versus dry microsites, respectively. Plants in dioecious populations occur at low densities, flower early in the season and are visited by generalist fly pollinators, whereas plants in hermaphroditic populations occur at high densities, flower when temperatures are higher and are visited by specialist bee pollinators (Barrett 1992; Case and Barrett 2001). On the basis of these findings, Barrett (1992) proposed that avoidance of inbreeding was an important selective factor in the establishment of dioecy, especially in situations where a change in habitat had resulted in a shift towards more generalist pollinators and increased selfing and inbreeding depression.

In higher-rainfall areas of south-eastern Australia, populations of *W. dioica* are subdioecious and contain male and female phenotypes and low frequencies of hermaphrodite phenotypes (5–20%, Barrett 1992; Barrett *et al.* 1999; Ramsey and Vaughton 2001; Jones and Burd 2001). Females are constant in their gender expression, whereas males are sexually labile and can express either male or hermaphrodite phenotypes in different years (i.e. inconstant males, Barrett *et al.* 1999; Ramsey and Vaughton 2001). We do not know whether the breeding system of hermaphrodites in current subdioecious populations differs from that of hermaphrodites in ancestral cosexual populations in which females originally established. However, the presence of hermaphrodites in subdioecious populations makes them ideal to study the role of inbreeding avoidance in maintaining separate sexes and provides possible insight into the evolution of gender dimorphism. In subdioecious populations, hermaphrodites are capable of setting seed following selfing (Ramsey and Vaughton 2001), but relative self-compatibility and floral biology have not been quantified.

Here we examine the self-compatibility and floral biology of hermaphrodites in a subdioecious population of *W. dioica*. First, we examine the effect of selfing and the magnitude of inbreeding depression on the seed fertility of hermaphrodites by comparing seed set and seed abortion of self-, cross- and

open-pollinated hermaphrodites and cross- and open-pollinated females. Second, we examine the degree of temporal overlap of sexual functions within hermaphroditic flowers and the likelihood of self-pollination by assessing floral longevity, stigma receptivity, patterns of anther dehiscence and pollen longevity. Finally, we examine whether pollen quantity limits seed set of hermaphrodites by assessing pollen deposition onto stigmas of open-pollinated hermaphrodites and females.

Methods

Study species and populations

We studied a subdioecious population of *Wurmbea dioica* spp. *dioica* (Colchicaceae) (Macfarlane 1980) in open woodland at Yan Yean, 30 km north of Melbourne (37°57'S, 145°10'E). Plants perennialize from an underground corm and produce a single inflorescence with one to eight flowers. Flowers have six white elliptic tepals (4–11 mm long) in a single whorl and each tepal has a conspicuous purple nectary located towards the base. Anthers are extrorse and dehiscence occurs longitudinally, while the gynoecium has three stigmatic lobes. Flowers open acropetally and all flowers may be open concurrently on plants (Macfarlane 1980; Barrett 1992; Vaughton and Ramsey 1998; Ramsey and Vaughton 2001). Flowering occurs in September and sex ratios over several years were c. 43% females, 50% males and 7% hermaphrodites (Ramsey and Vaughton 2001). Floral visitors collect nectar from flowers and include flies, bees and occasionally butterflies. Males produce more and larger flowers than females, whereas flowers of males and hermaphrodites are similar in size. Bees and butterflies respond to this dimorphism and visit polliniferous morphs more frequently, whereas flies are non-discriminatory in their foraging (Vaughton and Ramsey 1998; Ramsey and Vaughton 2001).

Self-compatibility

We determined the self-compatibility of hermaphrodites by assessing seed set and seed abortion in the first flower of self- ($N = 35$ plants), cross- ($N = 23$) and open-pollinated ($N = 30$) hermaphrodites and cross- ($N = 26$) and open-pollinated females ($N = 30$). We also assessed autofertility of hermaphrodites ($N = 15$). Plants to be hand-pollinated were excavated from the field before the first flower had fully opened, transplanted into pots and placed in an unheated and insect-free glasshouse. Cross-pollinated flowers on hermaphrodites were emasculated just before anthesis. After flowers had been open for 12 h, they were pollinated on three consecutive days by brushing stigmas with a mixture of pollen from several male plants. Flowers on females were cross-pollinated similarly. Self-pollinated flowers on hermaphrodites were pollinated by brushing stigmas with a recently dehisced anther from the same plant on three occasions. To assess autofertility of hermaphrodites, flowers were left untouched. Open-pollinated plants were marked in the field and after flowers had been open for 10 days they were excavated and transplanted into the glasshouse. After flowering, all plants were fertilised with half-strength liquid fertiliser and were watered regularly while the fruits matured.

We harvested the first fruit of plants when they were full-sized and counted the numbers of seeds, aborted seeds and ovules. Aborted seeds were shrivelled and ranged in size between ovules and seeds. We probably underestimated abortions because early aborted embryos could not be detected. We calculated percentage seed set as (number of seeds)/(number of aborted seeds + unfertilised ovules) and seed abortion as (number of aborted seeds)/(seeds + aborted seeds). We present percentage seed set rather than seed number because females produce more ovules per flower than hermaphrodites and we were interested in relative ovule–seed conversion (Ramsey and Vaughton

2001). We compared percentage seed set and abortion among treatments by 1-factor ANOVAs. In the autofertility treatment, hermaphrodites produced neither seeds nor aborted seeds and we did not include these plants in the analyses. We also assessed the relationship between seed set and abortion for self- and cross-pollinated hermaphrodites by linear regressions.

We estimated the relative performance of selfed to crossed hermaphrodites as $RP = w_s/w_c$, where w_s and w_c are the seed sets following self-pollination and cross-pollination, respectively. We could not use individual crossed seed-set values because pollination treatments were completed on different plants. Instead, we assessed variation in RP with individual selfed values and mean crossed seed set. This is justified because differences in seed set among crossed plants are likely to be related to spatial or resource constraints rather than genetic factors. We summarised inbreeding depression as $\delta = 1 - w_s/w_c$ (Charlesworth and Charlesworth 1987).

We estimated the zygote selfing rate of naturally pollinated hermaphrodites as $S = (p_c - p_o)/(p_c - p_s)$, where p_c is the mean percentage seed set after cross-pollination, p_s is the mean percentage seed set after self-pollination and p_o is the mean percentage seed set after open pollination (Charlesworth 1988). This procedure is based on the ability to measure a difference in viability of selfed and crossed embryos shortly after fertilisation and assumes that seed set of open-pollinated flowers is determined solely by the level of selfing and not by the quantity of pollen deposited on stigmas. These assumptions are valid for the present study on *W. dioica* (see results). Because we assessed seed set after self-, cross- and open-pollination on different plants, it was inappropriate to use the 'delta method' for estimating the variance of S outlined by Charlesworth (1988). Instead, we estimated bootstrap confidence intervals for S from 1000 bootstrap samples of each p_c , p_s and p_o obtained by resampling the original data (Chernick 1999). The bootstrap values were then randomly combined to obtain 1000 estimates of S , from which we calculated 95% confidence intervals. Reusch (2001) used a similar approach to estimate variance for the relative fitness of selfed progeny by using the inbreeding coefficient for *Zostera marina*, a marine angiosperm.

Floral biology

We assessed floral longevity and patterns of anther dehiscence in hermaphrodites by harvesting inflorescence stems in the field just as the first flower was opening. We placed stems in water on a laboratory bench ($N = 7$) and examined the first flowers every 12 h for the next 7 days. At each time point, we recorded the condition of the stigma, the number of dehiscent anthers, the presence or absence of nectar and the condition and colour of tepals.

Onset and duration of stigma receptivity and ovule viability of hermaphrodites was examined in the glasshouse by cross-pollinating flowers of a known age and scoring seed set. We randomly allocated plants excavated from the field to 0-, 3-, 5- and 7-day treatments ($N = 5$). As the first flower on each plant opened, the undehiscent anthers were removed and the stigma was pollinated at the appropriate time with fresh pollen from two to four males previously collected from the field. After flowering, all plants were maintained as described above. We harvested fruits when they were full-sized, and counted the numbers of seeds, aborted seeds and ovules. Percentage seed set was calculated as described above.

Longevity of pollen from hermaphrodites was examined by placing pollen of known age in 2.5% sucrose germination medium (Kearns and Inouye 1993). We collected inflorescence stems from the field as the first flower was opening and placed them in water on a laboratory bench ($N = 5$). Flowers were left until one to two anthers had dehiscent; the remaining undehiscent anthers were removed. A dissecting needle was used to sample pollen from the dehiscent anthers immediately (0 h) and subsequently on each day for the next 7 days. Pollen was placed on a

drop of germination medium on a microscope slide and incubated on moist filter paper in a petri dish. After 10 h, a drop of acetocarmine was added and a coverslip was applied. Transects across slides were viewed at $\times 40$ magnification and the number of germinated and ungerminated grains counted. The proportion of germinated pollen grains was calculated from 200 grains in each sample. The temperature in the laboratory ($21.5 \pm 0.5^\circ\text{C}$) was similar to field temperatures during the day, but was higher at night.

We observed that dehiscent anthers appeared to close at night and under cold, wet conditions in the field. The effect of similar conditions was assessed in the laboratory by placing hermaphrodites in a refrigerator ($5\text{--}7^\circ\text{C}$). Inflorescence stems were harvested from the field as the first flower was opening and placed in water in the laboratory ($N = 24$). Flowers were left until two anthers dehiscent on each flower. The remaining undehiscent anthers were removed. Half of the plants were placed in a refrigerator, while the other half were left on the laboratory bench ($21.5 \pm 0.5^\circ\text{C}$). After 12 h, the number of closed and open anthers was scored on all plants. For the refrigerated plants the condition of the anthers was scored again after 1 h on the laboratory bench. We repeated the procedure over the next 4 days.

Pollen deposition onto stigmas

We assessed pollen deposition under natural field conditions by marking females and hermaphrodites just as the first flower was opening ($N = 20$). We removed the stigmas after 8 days and placed each one on a small cube of glycerin gel with basic fuchsin on a microscope slide, which we squashed under a coverslip after heating (Kearns and Inouye 1993). The total number of pollen grains was counted at $\times 40$ magnification. We compared pollen deposition of females and hermaphrodites by a 1-factor ANOVA. We also counted ovules in flowers of females and hermaphrodites to compare pollen deposition with the number of ovules per flower ($N = 20$).

Statistical analyses

For ANOVAs, percentages were arcsine square-root transformed, which improved homoscedasticity and normality as established by Levene's and Shapiro-Wilk's tests, respectively. Unless otherwise stated, means (\pm s.e.) are given.

Results

Self-compatibility

No seeds were produced in the autofertility treatment, indicating that flowers did not self-pollinate autonomously. Hermaphrodites were partially self-compatible and relative performance of selfed to crossed hermaphrodites was 0.44 ± 0.04 . Percentage seed set differed significantly among the pollination treatments (Fig. 1; $F_{4,139} = 24.87$, $P < 0.001$). Percentage seed sets of self- and open-pollinated hermaphrodites did not differ from each other. Both were lower than seed sets for cross-pollinated hermaphrodites and for cross- and open-pollinated females, all of which did not differ from each other. In contrast, percentage aborted seeds of self- and open-pollinated hermaphrodites were significantly higher than those for cross-pollinated hermaphrodites and cross- and open-pollinated females (Fig. 1; $F_{4,139} = 17.94$, $P < 0.001$).

For selfed, but not crossed hermaphrodites, percentage abortion occurred at a range of developmental stages and was negatively related to percentage seed set

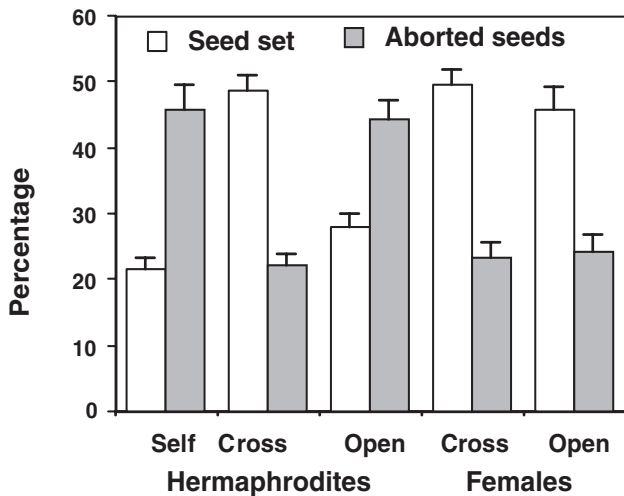


Fig. 1. Mean (\pm s.e.) percentage seed set and percentage aborted seeds of self-, cross- and open-pollinated hermaphrodites and cross- and open-pollinated females on *Wurmbea dioica* plants ($N = 23$ – 30 flowers per treatment). Values were determined from the first flower on plants. Percentage seed set and abortion of crossed hermaphrodites and crossed and open females did not differ (SNK tests, $P > 0.05$) and were greater and lesser, respectively, than that of selfed and open hermaphrodites (SNK tests, $P < 0.05$).

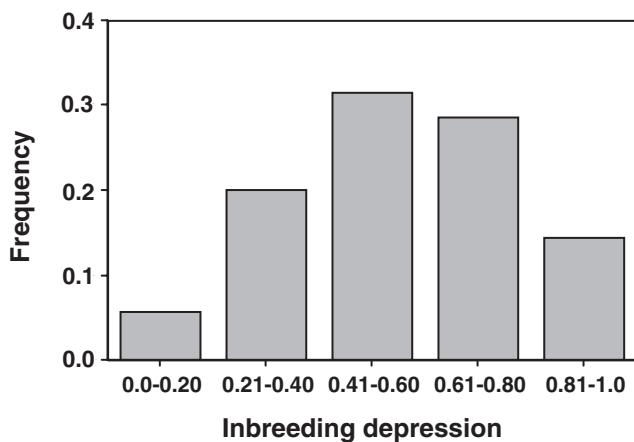


Fig. 2. Frequency distribution of inbreeding depression in *Wurmbea dioica* hermaphrodites ($N = 35$ plants). Inbreeding depression varied widely among plants and ranged from 0 to 1.0.

(self: $y = 67.3 - 0.9x$, $F_{1,33} = 11.15$, $P = 0.002$, $r^2 = 0.25$; cross: $y = 36.8 - 0.2x$, $F_{1,21} = 1.27$, $P = 0.273$, $r^2 = 0.05$). Percentage seed set following selfing also varied substantially (0–49%) among plants and the coefficient of variation of selfed plants was twice that of crossed plants (49.3 v. 23.6%). Inbreeding depression averaged 0.56 ± 0.04 and there was extensive variation among plants, ranging from 0 to 1.0 (Fig. 2). Despite high inbreeding depression, substantial selfing occurred in open-pollinated hermaphrodites ($S = 0.76 \pm 0.19$, mean \pm 95% confidence intervals).

Floral biology

Stigmatic lobes were reflexed and apparently receptive when flowers first opened and remained in that condition for at least 7 days. Anthers dehiscenced sequentially starting a few hours after flowers opened. On average, the length of time for all anthers to dehiscence was 3.7 ± 0.2 days. Nectar was observed on tepals following the dehiscence of the associated anther, indicating that flowers should be most attractive to pollinators when fresh pollen was available. Petals remained turgid and white for at least 7 days.

The periods of maximum pollen viability and stigma receptivity overlapped within flowers (Fig. 3). Seed set was 49% when flowers first opened and remained high for the next 5 days, before declining. Pollen germination was 80–90% when anthers first dehiscenced and remained high for the next 4 days, before declining.

Dehiscenced anthers closed when inflorescence stems were put in the refrigerator. The percentage of dehiscenced anthers that closed following refrigeration was 87, 65, 57, 30 and 17% after 1, 2, 3, 4 and 5 days, respectively. All anthers opened when stems were moved to the warmer temperature on the laboratory bench. Dehiscenced anthers on stems left permanently on the laboratory bench did not close but remained open continuously.

Pollen deposition onto stigmas

Pollen deposition onto stigmas of hermaphrodites and females did not differ significantly ($F_{1,38} = 0.70$, $P = 0.409$). Deposition exceeded the number of ovules per flower by about six-fold (pollen grains per stigma v. ovules per flower; hermaphrodites: 318 ± 24 v. 49 ± 3 , females: 356 ± 33 v. 60 ± 5), indicating that pollen quantity was unlikely to limit seed set.

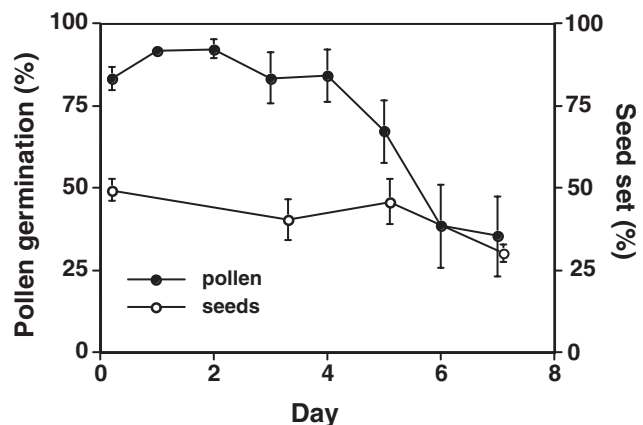


Fig. 3. Percentage pollen germination in germination medium ($N = 7$) and percentage seed set following cross-pollination ($N = 5$) with days after flower opening in *Wurmbea dioica* hermaphrodites. The periods of maximum pollen viability and stigma receptivity overlap during the first 5 days that flowers are open.

Discussion

In a subdioecious *Wurmbea dioica* population, hermaphrodites were moderately self-compatible (selfed seed set/crossed seed set = 0.44), but did not self-pollinate autonomously. Self- and open-pollinated hermaphrodites had equally low seed set and high seed abortion, which together with the high levels of natural pollen receipt indicate that seed set of open hermaphrodites was limited by pollen quality. By cross-pollinating flowers, we were able to increase percentage seed set and reduce percentage seed abortion of hermaphrodites to values comparable to those of open- and cross-pollinated females. Our non-genetic estimate of the zygote selfing rate of hermaphrodites following Charlesworth (1988) showed that appreciable selfing occurred under natural conditions ($S = 0.76$). Collectively, these results indicate that high selfing rates, usurpation of ovules by self pollen, and subsequent abortion probably due to early acting inbreeding depression severely limit seed set of hermaphrodites under natural conditions. We do not know whether the breeding system of hermaphrodites in subdioecious *W. dioica* populations differs from that of hermaphrodites in ancestral cosexual populations in which females originally established. However, self-compatibility and high inbreeding depression in hermaphrodites from this subdioecious population is consistent with the view that under current pollination conditions inbreeding avoidance is important in maintaining gender dimorphism.

Histological studies are required to determine whether low seed fertility following selfing is caused by late-acting physiological self-incompatibility (SI) or early acting inbreeding depression (Seavey and Bawa 1986; Manasse and Pinney 1991; de Nettancourt 1997). We suggest, however, that inbreeding depression and not SI is the likely cause for low seed set of open- and self-pollinated hermaphrodites. First, in selfed fruits many ovules are fertilised (c. 45%, M. Ramsey, pers. obs), indicating that levels of fertilisation following selfing are unlikely to limit fecundity. Second, in selfed fruits but not crossed fruits seed set was inversely related to seed abortion, and variation in seed set of selfed plants was twice that of crossed plants. Such patterns indicate that genetic load and inbreeding depression vary among plants, whereas with SI, selfed seed set is expected to be uniformly low. Third, aborted seeds from selfed fruits varied greatly in size, which is expected when different lethal recessive genes are expressed at different stages during seed development. With ovular SI, arrest is expected to occur at a similar and early stage of seed development. Together, these findings support our proposal that inbreeding depression rather than SI reduces seed set following selfing in *W. dioica* (Seavey and Bawa 1986; Wiens *et al.* 1987; Husband and Schemske 1996).

Our estimate of inbreeding depression at the seed stage ($\delta = 0.56$) was high compared with surveys of other

predominantly outcrossing populations (average of 40 populations, $\delta = 0.29$; Husband and Schemske 1996). Because *W. dioica* hermaphrodites are sexually labile and flower in most years as males (Barrett *et al.* 1999; Ramsey and Vaughton 2001), they may have little opportunity to purge their genetic load. We also found that inbreeding depression varied substantially among plants. Sakai *et al.* (1989) also found considerable variation in inbreeding depression at the seed stage in gynodioecious *Schiedea salicaria* ($\delta = 0-0.66$), although variation in other dimorphic species is lower (*Chionographis japonica*, $\delta = 0.11-0.24$, Maki 1993; *Schiedea adamantis*, $\delta = 0-0.30$, Sakai *et al.* 1997). In *W. dioica*, further studies are now required to assess levels of inbreeding depression at later life-cycle stages to determine the full impact of self-fertilisation on the relative fitness of hermaphrodites. The level of selfing and the magnitude of inbreeding depression in *W. dioica* will not only affect female frequencies in populations, but will also affect the relative fitnesses of constant versus inconstant males and the propensity for full dioecy to evolve.

Dichogamy, the temporal separation of sexual functions within flowers or among flowers on inflorescences, can play a major role in determining patterns of mating in self-compatible species (Schoen 1982; Harder *et al.* 2000). In *W. dioica*, we found evidence of weak dichogamy in hermaphrodites. Stigmas were receptive before pollen was shed, indicating that flowers were protogynous. However, temporal separation of sexual functions within flowers was very limited because anthers began to dehisce a few hours after flowers opened. Stigmas were receptive and pollen was viable for at least 5 days, and anther dehiscence occurred sequentially taking almost 4 days to complete. Such overlap between sexual functions within and among flowers would not prevent high levels of self-pollination by generalist fly pollinators, the major floral visitors (Vaughton and Ramsey 1998). Indeed, these traits would allow substantial opportunities for pollinator-mediated autogamy, and also geitonogamy because, although flowers open sequentially, most are open concurrently on plants.

In gynodioecious and subdioecious populations, polliniferous phenotypes achieve most of their fitness through male function, resulting in strong selection for effective pollen dispersal (Lloyd 1976, 1982). Sequential anther dehiscence in *W. dioica* would restrict the amount of pollen that can be removed by an individual pollinator during a single visit. Harder and Thomson (1989) proposed that sequential anther dehiscence is a response to diminishing male fitness returns as the amount of pollen removed by an individual pollinator increases. In *W. dioica*, this pattern of anther dehiscence should be beneficial because generalist fly pollinators are likely to forage haphazardly, removing pollen from anthers but often failing to visit flowers on other conspecific plants consecutively.

Selection should favour flowers that remain attractive to pollinators until pollen is removed from anthers and deposited onto stigmas (Ashman and Schoen 1996). Flowers of *W. dioica* remained attractive for at least 7 days as evidenced by turgid tepals and nectar production. This floral lifespan would in part be a response to the time required for the sequential opening of anthers. Other factors that would influence floral lifespan include prolonged pollen longevity and stigma receptivity, and closure of anthers in response to cold, wet conditions. Dafni and Firmage (2000) proposed that prolonged pollen longevity is expected when pollinator activity is unreliable or infrequent. Similar reasoning would pertain to prolonged stigma receptivity. Because flowering of *W. dioica* occurs in early spring, weather conditions are often unsuitable for pollinators for several consecutive days. Prolonged pollen longevity and stigma receptivity would reduce the risk of reproductive failure through either male or female functions. Similarly, closure of the anthers in cold, wet conditions could increase male fitness by reducing pollen wastage through exposure to the elements (Dafni and Firmage 2000).

Open-pollinated *W. dioica* females produce almost twice as many seeds as open-pollinated hermaphrodites and in part this is due to lower ovule production by hermaphrodites (Barrett *et al.* 1999; Ramsey and Vaughton 2001). In this study, we accounted for differences in ovule production by examining the percentage of ovules converting to seeds. Our results show that self-fertilisation, and subsequent abortion of selfed embryos due to early acting inbreeding depression, is a major factor limiting seed production of hermaphrodites under natural conditions. We also found a lack of temporal separation of male and female functions within hermaphroditic flowers, which would allow substantial opportunities for self-pollination mediated by insect pollinators. Overall, our findings corroborate Barrett's proposal (1992) that inbreeding avoidance is likely to have played a role in promoting the evolution of separate sexes in *W. dioica* populations, especially in situations where a habitat change has resulted in a shift in pollination mode towards more generalist pollinators.

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