Sexual dimorphism of dormancy and survivorship in buried seeds of *Silene latifolia*

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**Summary**

1. To test for differences in survivorship between buried male and female seeds in the dioecious perennial, *Silene latifolia* (Caryophyllaceae), the behaviour of large numbers of seeds buried under field conditions and unearthed after 5 and 10 months was compared with unburied seeds.

2. After 5 months of burial there was significant mortality of seeds and a large fraction of the remaining seeds displayed induced dormancy, i.e. they did not germinate under glasshouse conditions. The sex ratio of germinating seeds was not significantly different from the sex ratio of seeds germinated without burial (0 months).

3. Seed survivorship was constant from 5 to 10 months. However, by 10 months levels of dormancy had decreased so that most seeds germinated when unearthed and tested in a growth chamber. Furthermore, the proportion of females in germinating seed was significantly higher after 10 months than after 5 months.

4. Sex differences in mortality were therefore only revealed after 10 months, when dormancy had been broken. This result suggests that female seeds were more likely to become dormant during the first 5 months of burial, whereas males suffered greater mortality. Thus, sex differences in both survivorship and dormancy of buried seeds affected the sex ratio of germinating seeds over time. Such sexual dimorphism in seed bank dynamics may have an important effect on sex ratio variation in natural populations of dioecious plants.

**Keywords:** dioecy, seed bank, sex ratio

**Introduction**

Fisher (1930) demonstrated that the sex ratio of dioecious species should be maintained at 1:1 because genes that overproduce the minority sex will have higher fitness. An expectation of a sex ratio of unity is even stronger for species that possess sex chromosomes in which the males are XY because meiosis should produce equal proportions of X- and Y-containing gametes. Unequal numbers of males and females in populations of species with male heterogamy are therefore particularly noteworthy, and have prompted researchers to find the exact point in the life cycle when the bias develops. Determination of this stage is especially difficult in plants because the sex of an individual is only apparent upon flowering. For this reason, and due to an assumption that ecological differences between the sexes become large only during flowering, many studies on the development of sex ratio biases have focused on mortality of adult males and females (Lloyd & Webb 1977). Although differences have been noted in the success rates of X- and Y-containing pollen of several dioecious species (Correns 1928; reviewed by Lloyd 1974), it is not known whether other differences between the sexes exist prior to the onset of flowering. In light of the high rates of seed mortality in many species (Weaver & Cavers 1979; Roberts & Neilson 1980; Rees & Long 1993), the influence of sex differences in seed dormancy and survivorship may be particularly relevant to investigations of sex ratio biases in populations of dioecious plants.

Sex ratios of seedlings may be influenced by both seed dormancy and seed mortality. If the sexes differ in patterns of dormancy induction or germination responses to specific environmental cues, the sex ratio of germinating seedlings may vary. One type of dormancy, dark-induced dormancy, presumably minimizes nonadaptive germination at certain depths of
burial (Wesson & Wareing 1969; Thompson & Grime 1979; Pons 1991). Like other types of dormancy, dark-induced dormancy is often incomplete, and some seeds will germinate while buried (and then die, before the shoot can reach the surface). Similarly, when soil disturbance exposes seeds to optimal conditions, only some seeds will germinate. Both the frequency of induction of dormancy and the strength of the dormancy after soil disturbance may vary between the sexes. If these differences are strong, a change in sex ratio of the germinating seeds may occur and, in turn, affect the sex ratio of adult, flowering plants.

We addressed these issues with Silene latifolia (Carophyllaceae), a dioecious perennial of disturbed habitats in which populations are often female biased (Mulcahy 1967; Lovett Doust et al. 1987; Purrington 1994; but see Alexander & Antonovics 1988). Although a low rate of sex inconstancy has been reported after severe abiotic stress (Frick & Cavers 1989), sex ratio biases under normal circumstances require explanation. Recent glasshouse studies (Carroll & Mulcahy 1993; Purrington 1993) on this species have questioned the influence of differential fertilization success of X- and Y-containing gametes on progeny sex ratios, as proposed by Correns (1928), indicating that the existence of sex ratio biases in populations may reflect differences in mortality of males and females beyond the initial variation that has been found in the primary sex ratio of capsules (Lawrence 1963; van Nigtevecht 1966; Carroll 1993; Lyons et al. 1994; Taylor 1994a,b). Sex differences in adult mortality have not been demonstrated in S. latifolia (Alexander & Antonovics 1988), indicating that mortality at earlier stages in the life cycle may be more important in the establishment of sex ratio biases. We have therefore approached this issue with the prediction that large numbers of individuals may be removed from the population either temporarily through dormancy, or permanently through mortality. If these factors affect males and females differently, the potential for changes in the sex ratio of seedlings would be large. Specifically, we asked the following questions: (1) Are there differences in the proportion of males and females entering dormancy? (2) Are there sex differences in the strength of dark-induced dormancy? and (3) Do male and female seeds have different mortality rates while buried?

Materials and methods

Dispersal of seeds in Silene latifolia Poiret [= Silene alba (Miller)] is passive, with up to 500 seeds being produced within denticidal capsules. Seeds germinate all year, even during winter (personal observation), and the resulting plants are hardy perennials. While dispersal of seeds occurs throughout the summer and fall, many seeds remain within the capsules, which can become buried after the inflorescence stems decay. As a consequence, large groups of seeds from the same sibship often exist in close proximity within the seed bank (personal observation). Burial of seeds therefore will closely mimic the situation in which capsules become buried. Seeds for this experiment were collected from three crosses performed in the glasshouse in which three males, from bulk-collected seed from the Brown University Haffenreffer Preserve in Bristol, Rhode Island, were paired with three females from seeds collected in Virginia (by H. Alexander). This breeding design ensured that progeny sex ratios would not be affected by inbreeding with sibs, which might have been a problem had individuals from a single location been crossed. Multiple capsules were produced for each cross by hand pollination to produce large, full-sib families. Seeds were stored dry in foil-wrapped glass vials at room temperature for one month, and then at 4 °C for 2 months. Seeds were then placed within small bags (each containing 50 seeds) constructed of rayon mesh. On 11 December 1991, 20 bags of each family were buried 15 cm deep in a field at the Haffenreffer Preserve. Positions of families were randomized within an 8 x 10 grid, with each bag separated from its neighbours by 20 cm. A burial depth of 15 cm was chosen because it represents a depth at which emergence does not occur; depths of 4 cm or less are required for optimal establishment (Baker 1947). Twenty-four of these bags (eight from each family) were dug up on 19 May 1992 (5 months post burial) and the remaining 36 on 10 October 1992 (10 months post burial). Unearthed bags were washed free of soil and the seeds transferred to moistened filter paper in 5.5-cm Petri dishes. Petri dishes were placed in a growth chamber with 14-hour days (27 °C days, 19 °C nights) and watered as needed until seeds stopped germinating, approximately 30 days. At the end of this period the remaining seeds were examined to determine whether they were merely seed coat fragments. Intact seeds were considered still dormant rather than dead. (A tetrazolium test for viability was not performed on these putatively dormant individuals because seeds were subject to fungal infections after several weeks in the growth chamber, and testing after the 30 days would not have allowed inference about whether mortality had occurred in the soil or in the Petri dishes. This action was not likely to influence the results because the percentage of seeds maintaining dormancy at 10 months was ~ 0%). When individuals were identified as fragments of seed coats, it was assumed that they had germinated while still buried, and died. Germinating seeds were transferred from the Petri dishes to 6.5-cm plastic pots filled with Metro-Mix 360 and grown until flowering, at which time sex was recorded. The sexes of three individuals were determined by a PCR-based method using the RAPD Operon primer D12 as described in Mulcahy et al. (1992). One hundred seeds from each family were also germinated, without burial, in four replicate Petri dishes. This cohort of plants (the month 0 sample)
was grown to maturity in the Brown University greenhouse to establish the sex ratio under optimal conditions in which little seed mortality was expected. Three variables were computed for each bag: percentage surviving, percentage dormant, and percentage female of germinating seeds. Percentage surviving was defined as the percentage of intact seeds (i.e. the sum of those germinating and those remaining dormant upon testing).

Analyses of variance were performed on arcsine square-root transformed percentages treating duration of burial and family as fixed effects. Family was treated as a fixed effect because the three sibships were chosen nonrandomly on the basis of their large seed production, because large numbers of seeds were needed for the experiment. Nonorthogonal planned comparisons (0 vs. 5 months, 5 vs. 10 months, and 0 vs. 10 months) were performed with a adjusted to 0.017 due to nonindependence of the contrasts. All ANOVAs and contrasts were computed using SUPERANOVA (Gagnon 1990) with type III sums of squares.

Results

Germination of seeds in the month 0 cohort ranged from 91% to 100% for the three families, indicating low levels of innate dormancy in the stored seeds (Fig. 1a). Samples retrieved in May, after 5 months of burial, displayed significant mortality relative to the month 0 cohort (Fig. 1a, Table 1) although families differed in seed survivorship. Germination of the surviving seeds in the five month cohort was low (Fig. 1b, Table 1), indicating the presence of strong dark-induced dormancy in buried seeds. In October, after 10 months of burial, survivorship was not significantly reduced from the May sample (Fig. 1a, Table 1). There was, however, a significant decrease in dormancy from May to October (Fig. 1b, Table 1); in October almost all of the surviving seeds germinated, indicating that the induced dormancy observed after 5 months of burial had disappeared. Both survivorship and the level of dormancy over time differed among families as shown by significant duration by family interactions (Table 1).

In the month 0 sample, sex ratios were 50.5, 60, and 52.6% female for families A, B and C, respectively (Fig. 1c). Family B was slightly female-biased ($\chi^2 = 4.0, P = 0.05$) at this time. This observation cannot be attributed to differential emergence of males and females.

Table 1 Analysis of variance for survivorship, dormancy, and sex ratio in mesh bags containing seeds of Silene latifolia unearthed after 5 and 10 months of burial in a natural population. Percentage surviving = (no. surviving)/(no. buried); percentage dormant = (no. dormant)/(no. dormant+no. germinating); percentage females = (no. females)/(total no. germinating)

<table>
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<th>Source</th>
<th>d.f.</th>
<th>Mean squares</th>
<th>F-ratio</th>
<th>Mean squares</th>
<th>F-ratio</th>
<th>Mean squares</th>
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<td>Duration</td>
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<td>1.732</td>
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<td>2.485</td>
<td>167.344***</td>
<td>0.132</td>
<td>6.198**</td>
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<tr>
<td>Family</td>
<td>2</td>
<td>1.328</td>
<td>102.509***</td>
<td>0.031</td>
<td>2.086†</td>
<td>0.119</td>
<td>5.580**</td>
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<tr>
<td>Duration x family</td>
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<td>0.262</td>
<td>20.198***</td>
<td>0.126</td>
<td>8.480***</td>
<td>0.010</td>
<td>0.481</td>
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<td>Residual</td>
<td>63</td>
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<td>0.015</td>
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<td>0.021</td>
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Planned comparisons:

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<th>Mean squares</th>
<th>F-ratio</th>
<th>Mean squares</th>
<th>F-ratio</th>
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<td>0 vs. 5 months</td>
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<td>2.579</td>
<td>198.988***</td>
<td>1.583</td>
<td>106.629***</td>
<td>0.132</td>
<td>6.198**</td>
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<td>0 vs. 10 months</td>
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<td>3.246</td>
<td>250.456***</td>
<td>0.169</td>
<td>11.403**</td>
<td>0.071</td>
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<td>5 vs. 10 months</td>
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<td>1.194</td>
<td>4.876</td>
<td>328.530***</td>
<td>0.252</td>
<td>11.788**</td>
</tr>
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***P < 0.01, **P < 0.001, †P = 0.07.
females because there was no innate dormancy in this family (Fig. 1b, 0 months). The sex ratio of germinating seeds after 5 months of burial did not differ significantly from the 0 month cohort (Fig. 1c, Table 1) despite high levels of mortality and dark-induced dormancy in this sample. However, the proportion female in germinating seeds increased significantly from May to October (Fig. 1c). At 10 months all samples contained significantly more females than males ($\chi^2$ tests, $P < 0.05$), compared to at 5 months when all families showed sex ratios statistically indistinguishable from 1:1. Since there was no significant mortality between these two samples, this change must be related to the decrease in proportion of dormant seeds over this interval.

Discussion

Although it is likely that timing of seed dispersal, burial depth, and timing of disturbance will affect germination characteristics within natural populations, our results showed that the effect of seed burial on sex ratio in Silene latifolia seedlings can be large. Our data suggested that populations derived from seeds experiencing no or brief (< 5 months) burial may be relatively unbiased. In contrast, burial for longer periods may cause the population to show a large excess of females. Such a bias in a cohort of seedlings, of course, might be difficult to detect within a large population of older, established plants. Therefore, the effect of burial is predicted to be most important in stands derived solely from a persistent seed bank. Despite this caveat, it is possible that an initial bias will be maintained in many environments where rapid growth of conspecifics and other fast-growing weeds will produce dense vegetation shade under which additional germination is reduced. Therefore, our results provide a previously unexplored explanation for sex ratio variation in populations of S. latifolia.

The design of the experiment also allowed us to determine the mechanisms by which the sex ratio of our samples became altered. Since the sex ratio of the surviving seeds at 5 months was effectively hidden by a high proportion of seeds with dark-induced dormancy, the response of the 5 and 10 month samples must be considered simultaneously. At 10 months, dormancy of the majority of seeds was easily broken, revealing a sex ratio skewed heavily towards females. Since mortality (either from pathogens or premature, nonadaptive germination) of seeds was minimal between 5 and 10 months, we can infer that the sex ratio of the surviving seeds at 5 months was similarly female biased. This initial bias must have been due to mortality of male seeds between burial and 5 months. However, since we found that the sex ratio at 5 months (which reflects only the nondormant seeds) was not biased, we can further infer that this must be due to a disproportionate number of females maintaining dormancy upon transference to growth chamber conditions. These results indicate that the sex ratio of a newly recruited cohort from a persistent seed bank will depend upon the balance between mortality of males and the induced dormancy of females.

The demonstration of marked seasonality in germination requirements in S. latifolia by Roberts & Boddrell (1984) suggest that the timing of burial and of disturbance may cause additional variation in sex ratio dynamics of natural populations.

It is important to note that significant excesses of females are rare in pooled seeds collected from natural populations of S. latifolia (Mulcahy 1967; Gross & Soulé 1981; Taylor 1994a, but see Lyons et al. 1994), and that the sex ratio of seeds collected from different populations may be significantly heterogeneous (Lyons et al. 1994; Taylor 1994a). This variation has prompted examination of capsules generated in greenhouse breeding designs, which have established that at least some of the variation in progeny sex ratio can be attributed to maternal and paternal genetic sources (Lawrence 1963; van Nigtevecht 1966; Carroll & Mulcahy 1993; Lyons et al. 1994; Taylor 1994a,b). The sex ratio of a natural population therefore is likely to be strongly affected by the amount of genetic variation for progeny sex ratio present in a population. Without further experimentation it is impossible to assess the relative contribution of initial biases in progeny sex ratios and sex differences in seed characteristics to population sex ratios. We note, however, that the initial sex ratio of the seeds (the primary sex ratio) may accurately reflect the eventual adult sex ratio in populations where seed burial is less common.

This study does not address the adaptive significance of sex differences in germination cues. For nondioecious species (e.g. hermaphroditic) it is often thought that smaller seeds, which would be least likely to survive after germination at great depths, should have stronger dark dormancy (Thompson & Grime 1979; Venable & Brown 1988). Therefore, if on average males are heavier than females, females might be less likely to break dormancy while still buried. However, Purrington (1994) found exactly the opposite, with male seeds weighing less than female seeds. The ultimate cause of sex differences in dormancy is also unknown. One testable adaptive hypothesis is that males risk germinating while buried because eventual fitness (through pollen production) is not substantially reduced because of energy loss from subterranean growth. This may also explain why males emerge before females (Purrington 1993).

Traditionally, explanations for biases in species with nonlable sexes have tended toward two extremes: prezygotic mechanisms (e.g. sex ratio distorters) and adult mortality, the latter of which is predicted by considerations of the differential costs involved in pollen and seed production (Lloyd & Webb 1977). We have presented evidence for the importance of differential mortality after seed dis-
persal but prior to seedling establishment, a period that has received almost no attention in flowering plants (but see McClatchie 1992 for data on a liverwort). This neglect is less an indicator of the potential importance of seed banks but rather a consequence of the difficulty of studying the sex ratio of seeds whose sex cannot be immediately assessed (Bierzychudek & Eckhart 1988). The effects of even small differences in survivorship and dormancy of males and females could potentially cause dramatic changes in the sex ratio because the seed bank can be large relative to the number of established plants in many species (Wesson & Wareing 1969). An extension of this prediction is that in environments in which disturbance frequency is correlated with other conditions (e.g. moisture or elevation), the sex ratio of recruited seeds may be patchy. Several studies (Meagher 1980; Waser 1984; Dawson & Ehleringer 1993) have found that males and females are segregated into different habitats, and have implicated differential mortality of the sexes in the production of these patterns. The demonstration that male and female seeds have different dormancy and survivorship characteristics suggests that there may be potential for habit segregation of some species to operate prior to seedling recruitment.

Our results are also important for the interpretation of experiments utilizing seed families with poor germination success. Specifically, when germination percentage of a sample of seeds is low the sex ratio of the fraction of germinating seeds may be quite different from that of the remaining, dormant seeds. Although previous researchers have used caution in the past when interpreting the sex ratio data from such families (e.g. Conn & Blum 1981), it is now apparent that this concern is real. Indeed, Lyons et al. (1995), speculated that sex-linked differences in seed dormancy may have caused an association between germination percentage and sex ratio in several glasshouse experiments on _S. latifolia_. This phenomenon is also relevant to recent conservation efforts that attempt to stockpile seeds of rare plants for future reintroduction (Centre for Plant Conservation 1991). An important assumption behind these programs is that mortality of a given fraction of stored seeds each year will not significantly reduce the valuable genetic variation present in the original collection (Hamilton 1994). Our results reveal that another hazard to this effort may be the more rapid mortality of one sex of a dioecious species, a factor that would tend to exacerbate the reduction in genetic variation through a constriction in the number of possible mating pairs that could be arranged. Given the large number of rare plants that are dioecious, this concern warrants further attention.

More generally, it would be of interest to determine whether variation in dormancy and survivorship of seeds can affect sex ratios in other species with similar X/Y systems and in species with other mechanisms of sex determination. Furthermore, it might be productive to examine the role of seed bank dynamics on morph-type frequencies in species with different breeding systems, such as gynodioecy and heterostyly.

Acknowledgements

We thank S. Dudley, J. Waage, M. Bertness, L. Brooks, T. Meagher, J. Bergelson, D. Taylor, and K. Thompson for commenting on earlier versions of this paper. D. Rand kindly allowed the use of his thermocycler for RAPD analysis of sex. We also appreciate field and glasshouse care by R. Pacheco and F. Jackson as well as the seeds provided by H. Alexander. Funds were provided by NSF Dissertation Improvement grant BSR-911260 and NSF grant BSR-8906291 to J.S. and R. D. Wulff.

References


Sex differences in seed dormancy and survivorship


Received 7 November 1994
revised version accepted 22 February 1995