

Environmental influence on primary sex ratio in a dioecious plant

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Edited by May R. Berenbaum, University of Illinois at Urbana-Champaign, Urbana, IL, and approved May 16, 2008 (received for review February 27, 2008)

The proximity of mates can influence mating opportunities and the quantity and quality of offspring, especially in dioecious plant species. Progeny sex ratios modulated by environmental conditions is one of the most radical ways in which offspring quality may be influenced, yet it has rarely been reported in plants. A mechanism proposed to influence progeny sex ratios in dioecious plants involves competition between female- and male-determining microgametophytes (certation) as a result of variation in pollination intensity. However, the role of selective fertilization in dioecious plants is controversial and has not been demonstrated under field conditions. Here we investigate whether natural variation in the spatial arrangement of females and males influences pollination intensity and progeny sex ratios in the wind-pollinated herb *Rumex nivalis*. Based on previous experimental manipulation of pollination intensity in this species, we predicted that maternal parents in close proximity to males would produce more strongly female-biased progeny sex ratios. We tested this prediction in six alpine populations in Switzerland by measuring the distance between focal females and neighboring males and assessing pollen loads and seed sex ratios of maternal parents. In four of the six populations, females positioned in close proximity to males captured more pollen and exhibited more female-biased sex ratios. Our results demonstrate that demographic aspects of the maternal mating environment can influence progeny sex ratios. The most probable explanation for biased primary sex ratios in *Rumex* is selective fertilization resulting from pollen tube competition.

female-biased sex ratios | pollination intensity | selective fertilization

The spatial context in which reproduction occurs is of critical importance for plants because of their sessile habit. Most plants mate and disperse offspring locally, so that mating success is context-dependent and influenced by plant density and the phenotypic composition of neighborhoods (1–3). Dioecious species are especially sensitive to spatial structure and composition because of the restricted number of mating groups within populations. Female reproductive success can be influenced by male flowering density, depending on the extent of pollen dispersal (4–6). Patch density and the local sex ratio may also affect parental fitness through their influence on pollination intensity. The amount of pollen captured by stigmas could potentially affect both the quality of offspring and progeny sex ratios through gametophytic competition (certation) and selective fertilization (7, 8). However, the relative roles of genetic and environmental factors in governing primary sex ratios in dioecious populations are still poorly understood, and evidence for environmentally induced variation in primary sex ratios is limited despite considerable heterogeneity in seed sex ratios (9).

Sex determination induced by the environment is one of the most direct ways in which progeny sex ratios can vary. Environmental sex determination is expected to be adaptive if the environment experienced during development is variable and exerts a sex-dependent influence on fitness (10, 11). There are numerous examples in the animal kingdom in which environmental sex determination results in biased progeny sex ratios, with environmental triggers such as temperature or parental condition commonly involved (12–14). However, because plant

species are generally more plastic than animals in gender expression, they typically show environmentally influenced sex-allocation plasticity or sex inconstancy during flowering (15–17). Evidence for environmental sex determination early in development is relatively rare in seed plants (e.g., *Spinacia oleracea*: ref. 18), and how frequent environmental influences interact with genetic sex determination mechanisms to influence progeny sex ratios is unclear.

In the dioecious herb *Rumex nivalis* (Polygonaceae), sex determination is governed by heteromorphic sex chromosomes with females homogametic XX and males heterogametic XY₁Y₂ (19, 20). Similar to several other dioecious species of *Rumex* with sex chromosomes (7, 21–24), there is evidence that progeny sex ratios are also influenced by nongenetic factors, specifically the amount of pollen deposited on stigmas. By experimentally manipulating the distance between male pollen donors and female recipients of *R. nivalis* in a common garden, Stehlik and Barrett (25) demonstrated that seed sex ratios were dependent on the specific maternal pollination environment. Females at closer distances to males had higher stigmatic pollen loads and produced more strongly female-biased seed sex ratios compared with more distant females. However, attempts to demonstrate relations between male proximity and progeny sex ratios have been inconclusive in other *Rumex* species (23, 24), and the role of maternal pollination environment in affecting progeny sex ratios in natural populations has not been investigated.

Here we investigate whether the composition of the local mating neighborhood in natural populations of dioecious *R. nivalis* influences progeny sex ratios. Under the certation hypothesis, progeny sex ratios of maternal parents of *R. nivalis* located in close proximity to males should be more female-biased as a result of higher pollen loads, leading to increased competition between female- versus male-determining pollen tubes. We tested elements of this prediction in six natural populations of *R. nivalis* in the Swiss Alps. We mapped the location of plants in each population and measured pollen loads of females. Sex-specific molecular markers and censuses of flowering sex ratios of maternal families were then used to determine the sex of offspring. Our results provide evidence for an environmental influence on primary sex ratios in a dioecious plant.

Results

Variation in Population Size, Density, and Flowering Sex Ratios. The six populations varied in flowering population size (mean number of individuals = 1,145.8, SE = 163.1, range = 614–1,589) and flowering male density (mean males per square meter = 0.64, SE = 0.08, range = 0.12–1.26; Table 1). All populations were strongly female-biased (mean sex ratio = 0.75, SE = 0.03, range = 0.61–0.85; Table 1), a feature typical of *R. nivalis*

Author contributions: I.S., J.F., and S.C.H.B. designed research; I.S. and J.F. performed research; I.S. and J.F. analyzed data; and I.S. and S.C.H.B. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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Table 1. Summary of population parameters for six natural populations of dioecious *R. nivalis* in Switzerland used in the study of progeny sex ratio bias

Population	Geographic coordinates	Altitude, m above sea level	Population size	Male density, no. per m ²	Sex ratio	No. of focal females
Arosa1	9°37'7.2"/46°46'6.1"	2,500	1,333	0.98	0.61	25
Arosa2	9°37'7.3"/46°46'9.3"	2,250	614	0.12	0.85	21
Davos1	9°49'20.0"/46°41'32.3"	2,500	816	0.40	0.72	30
Davos2	9°48'33.7"/46°41'49.4"	2,330	1,542	1.26	0.77	29
Flims	9°16'21.8"/46°52'40.5"	2,500	981	0.53	0.78	26
Saentis	9°21'65.9"/47°14'30.1"	2,120	1,589	0.54	0.78	28

populations (26). As a result of variation in male density, there was a wide range of distances between focal females and surrounding males. In the case of the distance between a focal female and its fourth-nearest male (the independent variable in statistical analyses), the mean distance was 1.25 m (SE = 0.07, range = 0.24–6.7).

Pollen Load, Seed Set, and Seed Sex Ratios. The distance between a focal female and the fourth-nearest male had a significant effect on pollen capture in populations of *R. nivalis* (Fig. 1A and Table 2). Stigmatic pollen loads were largest in females with males in close proximity, whereas pollen loads decreased in females with male neighbors at further distances (Fig. 1A and Table 2). There was significant variation in pollen capture among the six populations (Table 2). This effect was partially due to significantly lower female pollen loads in Flims compared to other populations (partial regression coefficient, $b = -0.269$, SE = 0.208, $\chi^2 = 6.76$, $P < 0.05$), and Arosa2, in which pollen capture did not decrease with increasing distance to males (partial regression coefficient, $b = 0.512$, SE = 0.681, $\chi^2 = 0.57$, $P = 0.45$). We detected no pollen on 44% of sampled stigmas. The mean number of pollen grains per stigma for all females in the six populations was 1.65 (SE = 0.5). If we exclude stigmas that captured no pollen, the mean pollen load was 2.95 (SE = 0.08).

The distance between a focal female and its fourth-nearest male had a significant influence on seed set in *R. nivalis* (Fig. 1B and Table 2) despite high average seed set in all populations (mean = 0.83, SE = 0.01). Females in closer proximity to males set more seed than females farther away from males (Fig. 1B and Table 2). Additionally, there was a significant population effect and a significant interaction between population and distance to the fourth-nearest male (Table 2). This pattern was largely driven by Arosa1, in which seed set increased with increasing distance to males (partial regression coefficient, $b = 0.906$, SE = 0.254, $\chi^2 = 13.63$, $P < 0.001$).

The distance between focal females and their male neighbors had a significant influence on the degree of female bias in the progeny sex ratios of seeds (Fig. 1C and Table 2). Females with males in close proximity had the strongest female bias with decreasing bias with increasing distance between female and male plants (Fig. 1C and Table 2). There was a significant population effect and a significant interaction between population and distance to the fourth-nearest male (Table 2). These effects were largely due to the absence of association between seed sex ratios and distance to males for Davos1 (partial regression coefficient, $b = 0.014$, SE = 0.122, $\chi^2 = 0.01$, $P = 0.91$) and Arosa2 (partial regression coefficient, $b = 0.121$, SE = 0.126, $\chi^2 = 0.92$, $P = 0.34$). Fifty-seven of the 159 females produced significantly female-biased seed sex ratios, and no female produced a significant male bias. Overall, the mean progeny sex ratio was significantly biased toward daughters with a ratio of 0.65 (SE = 0.01; $\chi^2 = 9.76$, $P < 0.0001$).

There was a positive relation between seed sex ratio and pollen

load ($\chi^2_1 = 3.88$, $P < 0.05$; Fig. 2), as well as a significant effect of population ($\chi^2_5 = 13.99$, $P < 0.05$). Females with higher pollen loads produced more female-biased seed sex ratios than females with lower pollen loads, although there was considerable variation in this relation (partial regression coefficient, $b = 0.133$, SE = 0.068, $\chi^2 = 3.86$, $P < 0.05$; Fig. 2).

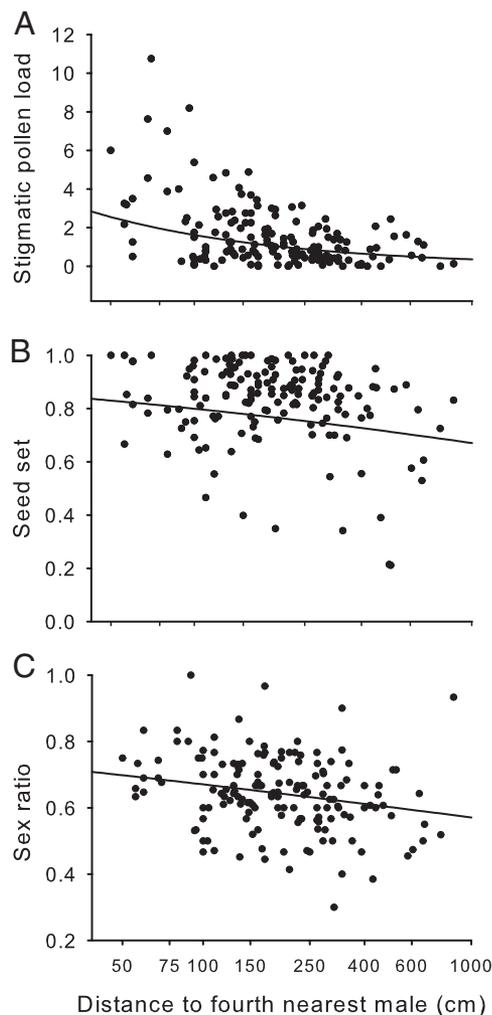


Fig. 1. The relation between stigmatic pollen loads (A), seed set (B), and seed sex ratios (C) of focal females and their distance to the fourth-nearest male in six populations of *R. nivalis* in Switzerland. See the text and Table 2 for statistical details. The predicted relations based on the generalized linear models are depicted. The equations for these relationships are as follows: $y = e^{3.51 - 0.66x}$ (A); $y = e^{2.38 - 0.14x} / (1 + e^{2.38 - 0.14x})$ (B); and $y = e^{1.56 - 0.18x} / (1 + e^{1.56 - 0.18x})$ (C).

Table 2. Summary of statistical analyses for the influences on stigmatic pollen load, seed set, and seed sex ratio for six natural populations of *R. nivalis*

Model	Sources of variation	Test results
Stigmatic pollen load	Population	$\chi^2_5 = 13.10^*$
	Distance to fourth-nearest male	$\chi^2_1 = 15.76^{***}$
	Population \times distance to fourth-nearest male	$\chi^2_5 = 11.38^*$
Seed set	Population	$\chi^2_5 = 110.78^{***}$
	Distance to fourth-nearest male	$\chi^2_1 = 7.46^{**}$
	Population \times distance to fourth-nearest male	$\chi^2_5 = 117.12^{***}$
Sex ratio	Population	$\chi^2_5 = 12.72^*$
	Distance to fourth-nearest male	$\chi^2_1 = 8.83^{**}$
	Population \times distance to fourth-nearest male	$\chi^2_5 = 12.57^*$

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Discussion

Our study demonstrates that the local pollination environment can influence progeny sex ratios in populations of a dioecious plant. Females of *R. nivalis* positioned in close proximity to males captured the most pollen (Fig. 1A), produced the highest seed set (Fig. 1B), and exhibited the most female-biased sex ratios (Fig. 1C). The most probable explanation for the association between higher stigmatic pollen loads and female-biased primary sex ratios (Fig. 2) is selective fertilization resulting from differential pollen-tube growth of female- versus male-determining pollen (certation). However, the role of certation in affecting sex-ratio variation in dioecious species is controversial (27–29), and previous attempts to demonstrate relations between pollination intensity and female bias under field conditions have failed (23, 24).

Pollen Dispersal and Female Bias in Seed Sex Ratios. We detected a significant bias toward female offspring in open-pollinated seed families of *R. nivalis* sampled from six populations in the Swiss Alps compared to the 1:1 ratio expected based on the sex determination system. No family exhibited an excess of males. The average frequency of females (0.65) in seed families was somewhat higher than we obtained in a survey of seed sex ratios in 18 Swiss populations (mean = 0.59; ref. 26) but within the range (0.74–0.63) that we recorded in a common garden experiment conducted at Toronto, Ontario, involving females positioned at different distances from males (25). Female-biased seed sex ratios have also been reported in other *Rumex* species. By manipulating the amount of pollen applied to stigmas of *Rumex acetosa* and *Rumex hastatulus*, Rychlewski and Zarzycki (23) and Conn and Blum (24), respectively, increased female bias

with heavier pollen loads. These results indicate that variation in pollination intensity can influence the degree of female bias in progeny sex ratios, and this appears to be a feature of the reproductive system of several *Rumex* species.

Wind dispersal of pollen typically follows a leptokurtic distribution with most pollen deposited near the source and a long flat tail characterized by low deposition (30, 31). Our previous experiment with small experimental arrays of *R. nivalis* indicated a steep decrease toward the flat end of the pollen dispersal curve (25). The range of distances used (5–150 cm) was shorter than we investigated in the field, where the mean distance between a female and her closest male was 92.7 cm (SE = 7.6). Pollen dispersal under natural conditions did not show as steep a decrease, probably because of the larger number of males available for pollen donation. Higher wind speeds in the Swiss Alps, in comparison with the earlier artificial array experiment, may have also played a role. In both studies no pollen was detected on a large proportion of stigmas that were sampled (44% in this study and 68%, in ref. 25). However, this was not reflected in a concomitant decrease in seed set in natural populations, which was generally high (mean = 0.83). Our stigma samples probably underestimated the total pollen loads that were captured because many stigmas were harvested before the duration of maximum longevity and pollen may have been washed off of stigmas during preservation in ethanol (see ref. 25).

The signal of a leptokurtic decrease in pollen capture with distance and its effect on female bias was evident in population comparisons. Arosa1, the population with the highest density of males (Table 1), showed the strongest decrease in female bias with increasing distance to males (mean distance between focal females and their closest males = 52.6 cm, SE = 1.3; partial regression coefficient in the generalized linear model, $b = -0.532$, SE = 0.216, $\chi^2 = 6.04$, $P < 0.05$), whereas Arosa2 and Davos1 had the lowest male densities and showed the weakest responses (mean distances for Arosa2 and Davos1 = 126.7 cm, SE = 7.5, and 110.7 cm, SE = 4.6, respectively; partial regression coefficients, $b = 0.121$, SE = 0.126, $\chi^2 = 0.92$, $P > 0.05$; $b = 0.014$, SE = 0.122, $\chi^2 = 0.01$, $P > 0.05$).

Effective wind pollination relies on efficient pollen removal and capture. This is best achieved in habitats unobstructed by vegetation and abiotic barriers, thus allowing the wind to move through the landscape in a laminar way (32, 33). *R. nivalis* was the dominant vegetation with no physical obstructions in most populations we sampled, including Arosa1. In contrast, nearby Arosa2 had the highest level of intermixing of *R. nivalis* with other alpine meadow plants, and the terrain was interspersed with large boulders. These differences in composition likely contributed to populations varying in their response of seed sex ratio to male distance, illustrating the potential role of ecological context in affecting progeny sex ratios.

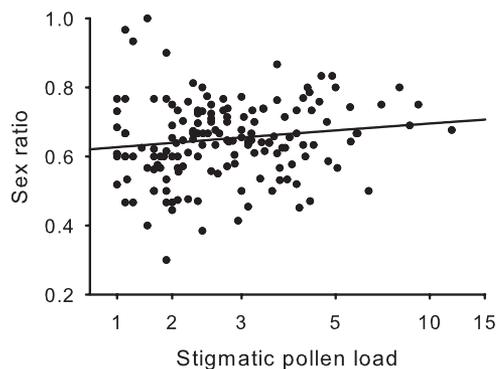


Fig. 2. The relation between the seed sex ratio and stigmatic pollen loads of females in six populations of *R. nivalis* in Switzerland. For statistical details refer to the text. The predicted relation based on the generalized linear model is depicted. The equation for the relationship is $y = e^{0.52+0.13x}/(1 + e^{0.52+0.13x})$.

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