Pollen limitation in an experimental population of the wild radish *Raphanus raphanistrum*

Karin S. Pfennig and Jeffrey K. Conner

Abstract: Experimental hand-pollinations were performed on a sample of wild radish, *Raphanus raphanistrum*, to determine whether female reproductive success was pollen limited. Fruit set was found to increase with receipt of supplemental pollen, but seed set did not. These results contradict findings in another *Raphanus* species but are expected if seeds are aborted or matured in packages.

Key words: pollen limitation, *Raphanus raphanistrum*, female fitness.

Résumé : Les auteurs ont conduit des pollinsations manuelles expérimentales sur des échanatillons de radis sauvage, *Raphanus raphanistrum*, dans le but de déterminer si le succès reproductif femelle est limité par le pollen. Avec un supplément de pollen, on observe une augmentation des mises à fruit, mais le nombre de graines demeure le même. Ces résultats ne concordent pas avec ceux trouvés chez une autre espèce, mais sont prévisibles si les graines avortent ou mûrissent dans leurs empaquetages.

Mots clés : limitation par le pollen, *Raphanus raphanistrum*, aptitude femelle.
[Traduit par la rédaction]

Introduction

Fruit or seed set may be limited in two ways: (*i*) insufficient resources (e.g., nutrients) are available to invest in progeny or (*ii*) insufficient pollen is available to fertilize ovules. Although both result in reduced female reproductive output, each mechanism has different implications for the evolution of floral traits in hermaphrodites (Johnston 1991*a*). When female fitness is limited by resources other than pollen, competition is expected to be high among males for the limited opportunities for paternity. In this situation, individuals will benefit from increased pollen removal, and selection is expected for traits that promote greater pollen dissemination (Pyke 1981; Johnston 1991*a*). If female fitness is limited by pollen availability, then selection is expected to favor characters that enhance pollen receipt (Johnston 1991*b*).

Pollen limitation is common: the majority of pollen limitation studies reviewed by Burd (1994) reported evidence of pollen limitation. The goal of our study was to determine whether female reproduction was limited by pollen availability in an experimental field population of the wild radish *Raphanus raphanistrum*.

Raphanus raphanistrum is a self-incompatible annual weed that was introduced from Europe at least 150 years ago (based on herbarium specimens; Panetsos and Baker 1967; J. Conner, unpublished data) and has become a model species for the study of floral evolution (Stanton et al. 1986; Mazer 1987; Snow 1990; Conner et al. 1996). Based on results for other species, we expected that pollen availability could be an important limitation on female fitness in our field population of *R. raphanistrum*. However, our population experiences very high rates of pollinator visitation (mainly by small bees and syrphid flies; Conner et al. 1996) and pollen removal (Rush et al. 1995). Furthermore, a previous study found no relationship between visitation and seed set in *R. raphanistrum* (Stanton et al. 1986), suggesting that female fitness often may not be pollen limited in this species.

Methods

Experimental hand-pollinations were performed in August 1992 to determine whether seed production was limited by pollen receipt. Twelve plants were chosen as pollen recipients out of a population of 60 located in a fenced plot at the University of Illinois Phillips Tract natural area. Pollen removal from flowers in this population averaged 84% in 1 h (Rush et al. 1995). Therefore, to ensure that plenty of pollen was available for application to the recipients' stigmas, four unrelated greenhouse-grown plants were used as pollen donors.

Each recipient was hand-pollinated with pollen from each of the four donors on four different days. On a given day, pollinations were performed at 2-h intervals five times throughout the day. At each pollination time, two racemes were chosen; on each raceme one flower was hand-pollinated and an adjacent flower was exposed to only natural pollination as a control. On one raceme, the control was the upper flower of the raceme, and on the other raceme it was the lower. Therefore, on each recipient plant there was a total of 2 pairs of pollinated and control flowers × 5 pollination times × 4 donors = 40 pairs of flowers. The number of fruits set by each control flower (0 or 1) was subtracted from the number of fruits set by the experimental flower for each pair of flowers. Because the flowers on a single plant are not independent, these differences were then averaged over all 40 flower pairs per recipient. A paired *t* test was then performed on the average differences for

Received February 27, 1996.

K.S. Pfennig[1] **and J.K. Conner.**[2] Department of Ecology, Ethology and Evolution, University of Illinois, 606 E Healey Street, Champaign, IL 61820, U.S.A.

[1] Author to whom all correspondence should be addressed. e-mail: kpfennig@email.unc.edu

[2] Present address: Kellogg Biological Station, Michigan State University, 3700 E Gull Lake Drive, Hickory Corners, MI 49069, U.S.A.

all 12 recipients because the average differences were continuously distributed. In cases where both flowers set fruit, the same analysis was performed using number of seeds per fruit in place of number of fruits. By doing separate analyses on fruit set and number of seeds per fruit, the potential effect of pollen limitation could be assessed on each of the two multiplicative components of total seed set.

Results

The hand-pollination experiment showed evidence for weak pollen limitation in fruit set but not seeds per fruit. The number of fruits set by flowers that were hand-pollinated was significantly greater than paired control flowers (mean difference hand-pollinated minus control = 0.06, paired t = 2.2, 11 df, $P < 0.05$). On average, 59% of hand-pollinated flowers set fruit versus 54% fruit set for control flowers. The number of seeds per fruit did not differ (mean difference hand-pollinated minus control = −0.01, paired t = 0.1, 11 df, P = 0.90); the mean number of seeds per fruit was 3.45 for both experimentals and controls.

Discussion

Our finding of pollen limitation of fruit set but not seeds per fruit is opposite to that reported in another *Raphanus* species, *Raphanus sativus* (Stanton 1987). The pattern we observed is expected, however, if ovules are matured or aborted as packages (Burd 1994). If the most efficient use of resources is to mature fruits containing full complements of seeds, then pollen limitation is expected to have the greatest effect on fruit maturation (Burd 1994). The difference between the two *Raphanus* species may point to different allocation strategies of resources to progeny. This possibility is an interesting area for future work.

A limitation of this study, shared with most studies of pollen limitation (Zimmerman and Pyke 1988), is that it fails to fully account for the possibility that resources other than pollen are limiting fruit production. The individual plants in our study may have been allocating scarce resources to those flowers that received the most pollen at the expense of other flowers on the same plant (Zimmerman and Pyke 1988). Plants in our experimental population produced an average of 683 flowers over their lifetimes, so the 40 flowers that we hand-pollinated represented only about 6% of the total number of flowers produced. A more effective design would be to compare the seed and fruit set on experimental plants that had received hand-pollination treatments of most flowers with control plants that had been exposed to only natural pollination (e.g., Zimmerman and Pyke 1988; Johnston 1991*a*). Even if our fruit-set results are due to differential allocation of resources due to pollen loads, this remains an interesting phenomenon worthy of further study.

Finally, Conner et al. (1996) found selection for increased flower size through differences in female fitness in this population, and Conner and Rush (1996) found that larger petal size increases pollinator visitation. Therefore, our evidence for pollen limitation provides a possible causal link between these findings. Experimental manipulation of flower size is necessary to test this possibility that larger flower size increases female fitness through increased pollen receipt.

Acknowledgments

We thank Rachel Neumeier for help with hand-pollinations and Scott Rush for help in collecting seeds.

References

Burd, M. 1994. Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. Bot. Rev. **60**: 83–139.

Conner, J.K., and Rush, S. 1996. Effects of flower size and number on pollinator visitation to wild radish, *Raphanus raphanistrum*. Oecologia, **105**: 509–516.

Conner, J.K., Rush, S., and Jennetten, P. 1996. Measurements of natural selection on floral traits in wild radish (*Raphanus raphanistrum*). I. Selection through lifetime female fitness. Evolution, **50**: 1127–1136.

Johnston, M.O. 1991*a*. Pollen limitation of female reproduction in *Lobelia cardinalis* and *L. siphilitica*. Ecology, **72**: 1500–1503.

Johnston, M.O. 1991*b*. Natural selection on floral traits in two species of *Lobelia* with different pollinators. Evolution, **45**: 1468–1479.

Mazer, S.J. 1987. The quantitative genetics of life history and fitness components in *Raphanus raphanistrum* L. (Brassicaceae): ecological and evolutionary consequences of seed-weight variation. Am. Nat. **130**: 891–914.

Panetsos, C.A., and Baker, H.G. 1967. The origin of variation in "wild" *Raphanus sativus* (Cruciferae) in California. Genetica (The Hague), **38**: 243–274.

Pyke, G.H. 1981. Optimal nectar production in a hummingbird pollinated plant. Theor. Popul. Biol. **20**: 326–343.

Rush, S., Conner, J.K., and Jennetten, P. 1995. The effects of natural variation in pollinator visitation on rates of pollen removal in wild radish, *Raphanus raphanistrum* (Brassicaceae). Am. J. Bot. **82**: 1522–1526.

Snow, A.A. 1990. Effects of pollen-load size and number of donors on sporophyte fitness in wild radish (*Raphanus raphanistrum*). Am. Nat. **136**: 742–758.

Stanton, M.L. 1987. Reproductive biology of petal color variants in wild populations of *Raphanus sativus*: II. factors limiting seed production. Am. J. Bot. **74**: 188–196.

Stanton, M.L., Snow, A.A., and Handel, S.N. 1986. Floral evolution: attractiveness to pollinators increases male fitness. Science (Washington, D.C.), **232**: 1625–1627.

Zimmerman, M., and Pyke, G.H. 1988. Reproduction in *Polemonium*: assessing the factors limiting seed set. Am. Nat. **131**: 723–738.