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Variation of pollen limitation in the early flowering Mediterranean geophyte *Narcissus assoanus* (Amaryllidaceae)

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Abstract A recent literature review indicates that pollen limitation of female fertility is a common feature of flowering plants. Despite the ecological and evolutionary significance of pollen limitation, most studies have only examined fertility in a single population at one time. Here we investigate pollen limitation of fruit and seed set in five populations of *Narcissus assoanus*, a self-sterile, insect-pollinated geophyte, over 2–3 years in southern France. In common with many early spring flowering plants, pollinator visitation to *N. assoanus* is often infrequent. Supplemental hand-pollination of flowers with outcross pollen significantly increased overall fruit and seed set by 11% and 19%, respectively. Four of the five populations experienced some pollen limitation during the study. For a given year, there was significant variation in pollen limitation among populations. Two of the populations were pollen limited in one year but not in other years in which they were studied. Seed:ovule ratios for open- and hand-pollinated flowers averaged 0.29 and 0.33, respectively. While hand pollination significantly increased the seed:ovule ratio, the low value obtained indicates that the majority of ovules in flowers do not mature seeds despite hand pollination. The role of genetic and environmental factors governing low seed:ovule ratios in *N. assoanus* is discussed.

Key words Fruit and seed set · *Narcissus assoanus* · Pollen limitation · Reproductive ecology · Seed:ovule ratios

Introduction

Plant fertility may be limited by resources (Bell 1985; McCall and Primack 1987) and/or pollen availability (reviewed in Burd 1994; Wilson et al. 1994). Although these factors are not mutually exclusive (e.g., Haig and Westoby 1988; Campbell and Halama 1993), it has often been assumed, following Bateman's (1948) principle, that pollen limitation of female reproductive success rarely occurs (see refs in Burd 1994). However, a recent literature review demonstrated that pollen limitation was evident in 62% of the species that have been examined (Burd 1994). Pollen limitation can be assessed in populations through comparisons of the fertility of open- versus hand-pollinated flowers (Bierzychudek 1981; Rathcke 1983). While the intensity of pollen limitation seems likely to vary with environmental conditions, few studies have examined the effects of supplemental hand pollination at both spatial and temporal scales (but see Dierhinger 1992; Alexandersson and Ågren 1996; Dudash and Fenster 1997). For the 258 species included in Burd's (1994) review, only 15% involved data from more than one site *or* year and only 4% were examined in multiple sites *and* years. Variable pollination environments are often invoked as an evolutionary force influencing mating and fertility, particularly in animal-pollinated species (reviewed in Harder and Barrett 1996). Hence information about spatial and temporal variation in pollen limitation may provide valuable information on the ecology and evolution of plant reproduction (Haig and Westoby 1988; Johnson and Bond 1997; Morgan and Schoen 1997).

When flowers receive insufficient pollen and not all ovules are fertilized, the ratio of seeds to ovules (hereafter S/O) is less than 1. In addition to pollen limitation, several other factors can influence variation in S/O ratios including resource limitation and genetic load (reviewed in Charlesworth 1989). A survey of S/O ratios in flowering plants indicated that outcrossing perennials exhibited significantly lower ratios than selfing annuals (Wiens 1984). Wiens interpreted this pattern as resulting from differences in genetic load between the two life histories.

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However, Wiens' survey of S/O ratios only involved data from open-pollinated flowers and it is therefore possible that the low S/O ratios reported in perennial plants also resulted from pollen limitation of seed set (and see Burd 1994). Comparisons of the S/O ratios of open- versus hand-pollinated flowers could provide insights into the relative importance of pollen limitation and genetic load as mechanisms responsible for reduced female fertility in flowering plants.

In most temperate regions, pollinator abundance generally increases during the growing season as a result of warmer temperatures in summer than early spring. Animal-pollinated species that flower in early spring therefore often experience pollen limitation owing to unreliable pollinator service (Schemske et al. 1978; Motten 1986; Barrett and Helenum 1987; McCall and Primack 1992). One of the most conspicuous early spring flowering genera native to the Mediterranean is *Narcissus* (Amaryllidaceae). Pollinator observations in populations of *Narcissus* species have often indicated that visitation rates are low (e.g., Fernandes 1965; Barrett et al. 1996). For example, in self-compatible *Narcissus longispathus*, unsuitable weather during February–April often limits pollinator activity and supplemental pollinations demonstrated that fruit set was pollen limited (Herrera 1995). Unlike *N. longispathus*, most *Narcissus* species are self-sterile (reviewed in Barrett et al. 1996) and are therefore likely to be especially vulnerable to unreliable pollinator service. Here, we investigate pollen limitation in self-sterile *N. assoanus* Léon-Dufour (section *Jonquillae*), a diminutive insect-pollinated species that flowers from mid February to late April in southern France and Spain.

N. assoanus is particularly suitable for studies of pollen limitation since populations are abundant and flowers are easily hand pollinated. In addition, the modal display size of individual plants in southern France where we conducted our study is a single flower. Therefore problems of interpretation associated with resource allocation among multiple flowers are not an issue (reviewed in Burd 1994). In this paper we report on the extent of pollen limitation of fruit and seed set in five natural populations occurring in the Languedoc-Roussillon region of southern France over a 3-year period. We were particularly interested in determining if there were differences among populations in pollen limitation for a given year and if the degree of pollen limitation in a population varied among years. We also examined whether supplement-

tal hand pollination increased S/O ratios in comparison with open-pollinated flowers.

Materials and methods

Study organism and populations

N. assoanus is a perennial geophyte widespread in southern France and eastern Spain (Blanchard 1990). It typically occurs in meadows and stony pastures on limestone from sea level to at least 1,900 m. In southern France, the solitary yellow flowers remain open for approximately 3 weeks (range 15–28 days), have prominent coronas, long floral tubes containing nectar, and are visited primarily by butterflies (*Gonepteryx cleopatra*), day-flying hawkmoths (*Macroglossum stellatarum*), and solitary bees (*Anthophora* spp.). *N. assoanus* is moderately self-sterile and all out-cross pollinations are compatible (Baker et al. 2000a).

The five populations in which we investigated pollen limitation were separated by a minimum distance of 8 km. Localities and information on altitude, flowering periods, and population size are presented in Table 1. Preliminary observations of pollinator activity in the five populations indicated that visitation rates were often low. During most of our visits to populations, no pollinators were seen because of inclement weather. However, we were able to quantify pollinator abundance on fine sunny days by recording the number of flower visitors entering 1- to 2-m² quadrats with an average density of 25 flowering plants/m² at 15-min intervals. The total number of observation periods per population ranged from 10 to 30 during the 3-year study. All populations except for the upland population at Rigalderie were located on stony, well-drained limestone substrate with south to southeast exposure. The Rigalderie population was located in an open pasture. At peak flowering, density was similar at all lowland sites (Aguzan, Col Hortus, Pont sur Brestalou, and St-Bauzille) with between 15 to 20 flowering individuals/m² on average. The density of flowering individuals was higher at Rigalderie (approx. 50/m²). In all populations, flowering periods lasted for approximately 4–6 weeks.

Measurement of pollen limitation of fruit and seed set

We investigated spatial and temporal components of pollen limitation of fruit and seed set in the five populations in 1997–1999. Aguzan, Col Hortus, and St-Bauzille were studied in every year. Pont sur Brestalou and Rigalderie were each examined in only 2 years because of insect herbivory and poor weather conditions, respectively. We began pollination treatments circa 1–3 weeks after flowering began in each population. By 3 weeks, all populations were in peak flower. We set up a series of five to eight 1- to 2-m² plots in each population. Within these plots flowering density was approx. 25/m² and we used flowers in these plots for our study. Flowers were chosen 1–2 days after opening and supplemental outcross pollen from a single pollen donor was brushed onto the stigmas of the flower using fine-tipped forceps. Pollen donors were chosen from 1 to 5 m away. Because of the high density of flowering plants, this ensured that crosses were not between near neighbors. Other flowering individuals in the plots were marked

Table 1 Populations of *Narcissus assoanus* in southern France examined for pollen limitation of fruit and seed set. Pollinator abundance was measured as the proportion of all observation periods in which pollinators were seen

	Population				
	Aguzan	Col Hortus	P. sur Brest	Rigalderie	St-Bauzille
Longitude	03°53'10" E	03°51'20" E	03°58'50" E	03°30'20" E	03°57'30" E
Latitude	43°56'20" N	43°48'55" N	43°50'45" N	43°55'45" N	43°46'20" N
Altitude (m)	200	240	150	680	220
Peak flowering	Mid March	Mid March	Mid March	Mid April	Mid March
Pollinator abundance	0.67	0.37	0.46	0.20	0.82
Population size	1,200	1,200	400	10,000	600

with white tape at the base of the flowering stem and left as open-pollinated controls. *N. assoanus* possesses a stigma height dimorphism (Baker et al. 2000a, 2000b). Plants of the long- and short-styled morph were both chosen for pollination treatments in each population. To facilitate pollination of the short-styled morph, the perianth was slit with forceps. This procedure was also applied to the long-styled morph. Flowers were left undisturbed after pollination, allowed to senesce, and seed capsules were collected when mature (4–5 weeks after anthesis). Seed number and the number of ovules per flower were measured using a dissecting microscope. An earlier study of morph-specific fertility in these populations indicated that there was no significant difference in fruit or seed set between the style morphs (Baker et al. 2000a). Consequently, in all analyses here we used pooled data and did not consider the effects of style morph on fruit or seed set.

Statistical analyses

Percent fruit set was analyzed with logistic regression (PROC GENMOD; SAS 1997) with population nested within year, year, and pollination treatment as main categorical effects. The interaction of year-by-pollination treatment examined the intensity of pollen limitation among years whereas the term pollination treatment-by-population nested within year investigated the degree of pollen limitation among populations for a given year. This analysis did not allow us to assess whether or not the intensity of pollen limitation differed among years for a given population. To address this question, we performed *G*-tests of independence (Sokal and Rohlf 1995) for each of the population-by-year combinations. We used the Dunn-Sidak correction to adjust the experimentwise error rate to account for multiple comparisons (Sokal and Rohlf 1995). This correction is conservative and lowers the chance of making type I errors in the entire series of tests. Two approaches were used to examine the effects of population, year, and pollination treatment on seed set. In an analysis similar to the one above, we used a mixed-model ANCOVA (PROC GLM; SAS 1997) to examine the effects of the above variables on the squareroot of seed set per fruit. This transformation increased the normality of the data. In this analysis, year was treated as a random variable. To assess pollen limitation among years for a given population, we performed orthogonal contrasts (Student's *t*-test; Kirk 1995) to compare seed set in open- versus hand-pollinated capsules in the 13 population-by-year combinations. We back-transformed least-square means for presentation in figures. In a second analysis, we used a fully factorial mixed-model ANCOVA and the type IV sum of squares (PROC GLM; SAS 1997). This analysis accounted for the missing population-by-year combination allowing us to compare the results of this factorial design with the nested design described previously. We calculated S/O ratios by dividing the number of seeds in a capsule by the total ovule number (seeds+ovules). The distributions of S/O ratios for open- versus hand pollination treatments were compared using a Kolmogorov-Smirnov two-sample test (Sokal and Rohlf 1995).

Results

Pollen limitation of fruit set

Overall, supplemental hand pollination (HP) of *N. assoanus* flowers significantly increased percent fruit set by 11% in comparison with open-pollinated (OP) flowers (grand mean OP=66.1, SE=1.6, $n=821$; HP=73.7, SE=1.9, $n=585$; Table 2). The intensity of pollination limitation of fruit set did not differ among years or among populations within a given year (Table 2). Although no significant interactions between pollination treatment and year or population were found, *G*-tests of

Table 2 Logistic regression analysis of the effects of year, population, pollination treatment, and their interactions on percent fruit set in populations of *N. assoanus*

Source	<i>df</i>	χ^2	<i>P</i>
Year	2	4.69	0.0958
Population[year]	10	182.91	0.0001
Treatment	1	12.11	0.0005
Year×treatment	2	3.94	0.1394
Treatment×population[year]	10	14.21	0.1638
Error	1,380		

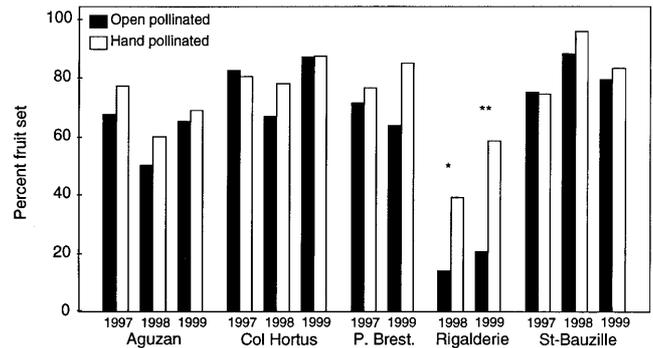


Fig. 1 Percent fruit set in open-pollinated (OP) vs hand-pollinated (HP) plants in five populations of *Narcissus assoanus*. Aguzan, Col Hortus, and St-Bauzille were studied in 1997–1999. Pont sur Brestalou and Rigalderie were studied in 2 years. Overall, there is a statistically significant difference in percent fruit set between OP and HP plants. Significant increases in percent fruit set with hand pollination are indicated by asterisks (** $P<0.01$, * $P<0.05$; following Dunn-Sidak's correction for multiple comparisons) and are based on *G*-tests of independence. Mean sample sizes are 63.4 (range 14–97) and 45.0 (range 10–79) plants for OP and HP, respectively

independence revealed significant pollen limitation of fruit set at Rigalderie in 1998 and 1999. However, in the other four populations (Aguzan, Col Hortus, Pont sur Brestalou, and St-Bauzille) hand pollination did not significantly increase fruit set in any year (Fig. 1). Rigalderie also exhibited significantly lower fruit set than the four lowland populations.

Pollen limitation of seed set

The results of the fully factorial design did not differ qualitatively from the nested design (data not shown). Here we present the results of the nested ANCOVA to maintain symmetry of interpretation with the results of the fruit set analysis. Hand-pollinated flowers of *N. assoanus* produced significantly more seeds (19%) than open-pollinated flowers (OP=12.94, upper SE=0.429, lower SE=0.422, $n=532$; HP=15.92, upper SE=0.480, lower SE=0.473, $n=399$; Table 3). The intensity of pollen limitation differed among populations within a given year, as indicated by the marginally significant interaction of pollination treatment-by-population nested within year (Table 3), but did not differ among years (as in-

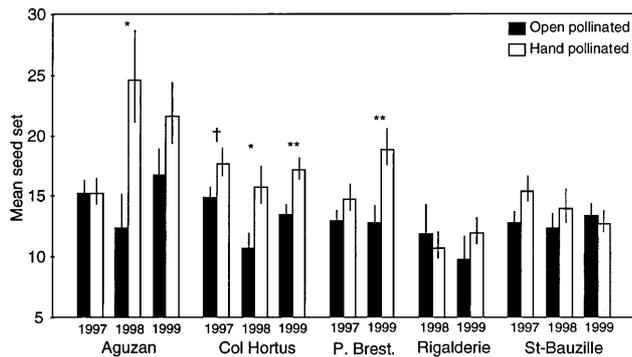


Fig. 2 Mean seed set in open- (OP) vs hand-pollinated (HP) plants in five populations of *N. assoanus*. Aguzan, Col Hortus, and St-Bauzille were studied in 1997–1999. Pont sur Brestalou and Rigalderie were studied in 2 years. Overall, there is a statistically significant difference in mean seed set between OP and HP plants. Population-by-year combinations with significantly higher mean seed set in HP flowers are indicated (** $P < 0.01$, * $P < 0.05$, † $P < 0.10$; following Dunn-Sidak's correction for multiple comparisons) and are based on orthogonal contrasts. Mean sample sizes are 40.9 (range 7–80) and 30.7 (range 6–60) plants for the OP and HP treatments, respectively. Error bars are equivalent to ± 1 SE

Table 3 ANCOVA of the effects of year, population, treatment (open vs hand pollinated) and their interactions on mean seed set per flower in populations of *N. assoanus*. Total ovule number was used as a covariate

Source	df	MS	F	P
Year	2	0.772	0.80	0.4484
Population[year]	10	3.536	3.68	0.0001
Treatment	1	21.090	21.93	0.0001
Year×treatment	2	1.224	1.27	0.2860
Treatment×population[year]	10	1.703	1.77	0.0619
Total ovule number	1	134.15	139.48	0.0001
Error	904	0.962		

indicated by a non-significant F -value for the year-by-pollination treatment interaction, Table 3). The results of orthogonal contrasts (t -tests) to examine seed set in open- versus hand-pollinated plants for each population-by-year combination indicated that there was variation in pollen limitation; seed set was pollen limited in only 1 year at Aguzan and Pont sur Brestalou but was never pollen limited at Rigalderie or St-Bauzille (Fig. 2). Seed set was pollen limited in every year at Col Hortus (Fig. 2), although in 1997 the difference between the treatments was only marginally significant.

S/O ratios

The mean S/O ratio pooled across all populations and years was significantly higher in hand-pollinated plants than in open-pollinated plants (OP=0.287, SE=0.007; HP=0.332, SE=0.008; $t_{929}=4.495$, $P < 0.001$; Fig. 3). Nevertheless, on average, two-thirds of the ovules in hand-pollinated flowers failed to mature seed. We pooled S/O ratios from the five populations because there

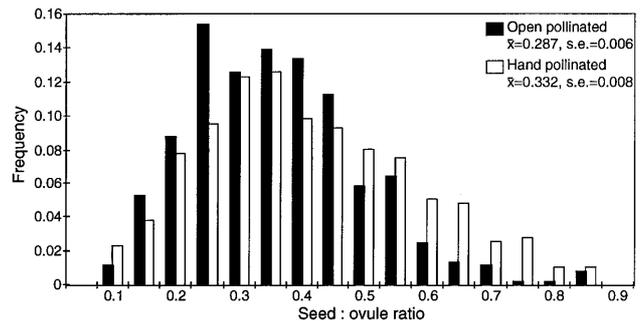


Fig. 3 Distributions of seed:ovule ratio in open- vs hand-pollinated plants of *N. assoanus*. Data were pooled from five populations over 3 years. The two distributions are significantly different following a two-sample Kolmogorov-Smirnov test ($D=0.144$, $P < 0.001$). Sample sizes are OP=532 fruits, HP=398 fruits

was no significant population-by-pollination treatment interaction following ANOVA ($F_{4,920}=1.54$, $P=0.1882$). Although distributions of S/O ratio for the OP and HP treatments were significantly different ($D=0.144$, $P < 0.001$; Fig. 3), both exhibited a wide range of values from 0.1 to 0.9.

Discussion

The major finding of this study is that populations of *N. assoanus* in southern France exhibit significant spatial variation in pollen limitation. Supplemental hand pollinations increased overall fruit and seed set by 11% and 19%, respectively. Four of the five populations experienced pollen limitation of fertility at some time. Of these, two populations were pollen limited in 1 year but not in the other years in which they were sampled. This variation cautions against generalizations about pollen limitation based on limited sampling of a single population. A second significant and unexpected result from our study was the finding that S/O ratios in all populations following hand pollination were surprisingly low, with only a third of ovules, on average, producing seeds. Below we discuss some of the factors influencing female fertility in *N. assoanus*, outline their ecological and evolutionary implications, and conclude by reviewing some of the mechanisms influencing S/O ratios in flowering plants.

Causes of pollen limitation

Among the five populations of *N. assoanus* that were sampled, 66% of open-pollinated flowers set fruit. Supplemental hand-pollination increased fruit set to 74%. Despite this overall increase, in only one population, Rigalderie, was fruit set significantly pollen limited. This indicates that in most populations, flowers of *N. assoanus* are visited by pollinators despite their low activity during parts of the flowering period. Flowers of *N. asso-*

anus have long floral longevities, ranging from 15–28 days depending on temperature and whether they have been pollinated. This extended floral lifetime undoubtedly increases the probability of visitation and accounts for the absence of significant pollen limitation of fruit set in most populations. Elsewhere, Primack (1985) and Ashman and Schoen (1996) provide empirical and theoretical evidence that extended floral longevities are a common adaptive feature of animal-pollinated species with infrequent pollinator service. Long floral longevities occur in most *Narcissus* species, particularly those that flower at times of the year when pollinator densities are low (Arroyo and Dafni 1995; Herrera 1995; Barrett et al. 1996).

The detection of pollen-limited fruit set in both years at Rigalderie likely stems from two primary causes. First, this population is located on an upland plateau at higher altitude and flowers 1 month later than the remaining populations. During flowering, this location often experienced cool windy conditions that limited pollinator activity during the study period. Poor weather, including low temperatures, nighttime frost, and snow may also adversely influence fruit set (in open- and hand-pollinated plants) when compared to lowland sites. At this site, flowering occurred during cooler weather in both years of our study. Second, this population is considerably larger than the other populations investigated, containing at least 10,000 flowering individuals (Table 1). Low fruit set at Rigalderie may also be associated with an excess of flowers relative to the number of pollinators visiting the population. Recent studies that have focused on the proximate ecological mechanisms governing plant reproductive success have tended to emphasize the negative effects of small population size on fertility because of the effects of reduced floral displays on pollinator visitation rates (e.g., Jennersten 1988; Ågren 1996; Alexandersson and Ågren 1996). However, the opposite pattern could also occur in very large populations where mean fertility may be reduced in comparison with smaller populations because of a surplus of floral resources (Fritz and Nilsson 1994; Larson and Barrett 1999). Such effects may be especially likely during unpredictable weather in early spring when pollinator densities may not track rapid changes in flowering density.

Pollen limitation of seed set most often arises because an insufficient number of outcross pollen grains are deposited on stigmas by pollinators. Seed set was pollen limited in four of the five populations of *N. assoanus* but did not occur in every year. Interestingly, while three of the four populations exhibited pollen limitation of seed set, at no time was fruit set in these populations limited by pollen delivery. This indicates that in general, sufficient pollen was transferred to stigmas to enable fruit maturation but not to ensure full seed set. Unlike many perennial plants that commonly abort fruit with small numbers of developing seeds (reviewed in Stephenson 1981), plants of *N. assoanus* maintain developing fruits even when they contain very few seeds (<5). This feature of the reproductive biology of *N. assoanus* may reflect

the low flowering capacity of individual plants which at our sites produce only a single flower each season. This contrasts with many mass-flowering trees and shrubs that typically exhibit high rates of fruit abortion. Although most flowers of *N. assoanus* matured fruit regardless of seed number, a significant proportion (26%) did not, even when they were provided with abundant pollen through hand cross-pollination. As discussed in more detail below in connection with the low S/O ratios, the causes of this fruit failure are not known but could involve both genetic and environmental factors.

Because of the generally low and unpredictable nature of pollinator service to *N. assoanus* populations, any attempt to correlate pollinator abundance to variation in pollen limitation is fraught with difficulty. This problem was also encountered by Larson and Barrett (1999) in their studies of pollen limitation in *Rhexia virginica*, a buzz-pollinated species that experiences low and unpredictable pollinator service at the geographic margins of its range. Our efforts at measuring pollinator visitation in *N. assoanus* were crude at best and provided only qualitative information on the relative abundance of pollinators at the five sites. The problem of quantifying pollinators is a general problem for animal-pollinated species with extended floral longevities and low visitation rates since it requires considerable time and effort, particularly when population comparisons are involved. Assays of pollen loads on stigmas and pollen removal from anthers may, however, provide indirect information on the activities of pollinators and enable inferences to be made on the role of variation in pollinator abundance in causing pollen limitation (Ackerman 1989; Kearns and Inouye 1993; Johnson and Bond 1997; Larson and Barrett 1999).

Why are S/O ratios so low?

Flowers of *N. assoanus* that were hand pollinated with outcross pollen matured only 33% of their ovules. This value is considerably lower than the mean S/O ratio (0.50) reported from a survey of open-pollinated perennial plants by Wiens (1984), raising the obvious question as to why S/O ratios in *N. assoanus* are so low. Although we presented pooled data on S/O ratios in Fig. 3, all five populations exhibited similar distributions, with mean values for hand-pollinated flowers consistently low (range of mean S/O values 0.271–0.370). This result suggests that the low S/O ratios we report are a general feature of this species. Next we consider potential factors that might explain this pattern.

Limited resources could contribute towards the low percent of ovules that mature into seed and also to the submaximal fruit set we recorded in hand-pollinated plants. The habitats that *N. assoanus* populations occupy are often dry and stony, and during fruit maturation are frequently subject to drought. We have some evidence that resource limitation plays a role in the low fertility of this species. In 1997, plants from several natural popula-

tions in this region were transplanted just prior to flowering to glasshouses at Montpellier for controlled pollination studies reported elsewhere (Baker et al. 2000a). These plants received regular watering and nutrients and were used to perform within-population hand cross-pollinations. Mean S/O ratios were significantly higher in these experimental plants (S/O ratio=0.423, SE=0.021, $n=151$) than the values obtained in this study for the same year (S/O ratio of HP plants in the field=0.335, SE=0.014, $n=100$; $F_{1,245}=12.157$, $P=0.0006$), although they were still well below the average values for most perennial plants (see Wiens 1984). This suggests that while resource limitation plays some role in regulating fruit and seed set in *N. assoanus* under field conditions, other factors are also involved.

Several aspects of our hand pollinations of *N. assoanus* could also have contributed towards reduced fertility. Both maternal and paternal genotype and their interaction play a role in governing the fertility of cross pollinations (Lyons et al. 1989). Our crosses involved single pollen donors occurring within 1–5 m of the maternal parent. It is possible that despite our efforts to avoid near neighbors, our crosses still involved related individuals and the low mean fertility we obtained reflects a component due to inbreeding depression (Charlesworth and Charlesworth 1987). This is certainly a distinct possibility, since *N. assoanus* populations are highly structured and seed dispersal is limited because of the diminutive status of plants. The inbreeding hypothesis could be tested by investigating the fertility of crosses at different spatial scales (Waser 1993). It is also possible that by increasing the number of male parents used in crosses fertility could have been increased (Marshall 1991). Another intriguing possibility concerns ovule maturation schedules within flowers of *Narcissus* species. In *N. triandrus*, an early flowering species with extended floral longevity, ovules mature asynchronously so that on a given day early in anthesis not all ovules are receptive (Sage et al. 1999). If this occurs in *N. assoanus*, then our hand pollinations conducted on a single day may not have been sufficient to fertilize most ovules within an ovary. This hypothesis predicts that serial pollinations over the lifetime of a flower should increase S/O ratios in *N. assoanus*.

Wiens (1984) explained the low fertility of outcrossing perennials as resulting largely from genetic load. According to this hypothesis, many fertilization products are subsequently aborted during early development because of deleterious allelic combinations. This hypothesis is difficult to evaluate since, as in many investigations (see Nakamura and Stanton 1987), we were unable to determine whether ovules in fruits of *N. assoanus* had been fertilized and then aborted very early in development or whether they had remained unfertilized. Charlesworth (1989) suggested that genetic load could provide a partial explanation for low S/O ratios although she doubted that load alone could account for the low fertility observed in some plants. To critically evaluate genetic hypotheses that account for low fertility in outcrossing

species, both fertilization rates and the viability of female and male gametes must be determined. In particular, the assumption that most ovules are viable needs to be verified by careful cytological examination.

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