Spatial ecology of mating success in a sexually polymorphic plant

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The spatial context of reproduction is of crucial importance to plants because of their sessile habit. Since pollen and seed dispersal is often restricted, mating success is likely to depend on the quantity and quality of mates in local neighbourhoods. Here we use neighbourhood models to investigate the spatial ecology of pollination and mating in *Narcissus assoanus*, a sexually polymorphic plant with two mating morphs that differ in style length. By mapping individuals in eight populations from southwestern France, we investigated the influence of the density and morph identity of plants at different spatial scales on variation in female fertility. By using inferences on the expected patterns of pollen transfer based on floral morphology, we were able to predict the quantitative relations between local morph ratios and variation in fertility. Our analyses revealed differences in the spatial clustering of morphs and in their response to plant density and morph identity within local neighbourhoods. Mating success in *N. assoanus* was characterized by both density- and frequency-dependent processes, a condition that may be a general feature of the spatial ecology of plant mating.

Keywords: mating success; neighbourhood model; style-morph ratios; sexual polymorphism; spatial pattern

1. INTRODUCTION

Most flowering plants mate and disperse offspring locally because of their sessile habit. As a consequence, the density and spatial distribution of individuals within populations play an important role in determining variation in reproductive success (Levin & Kerster 1969; Antonovics & Levin 1980; Kunin 1993; Groom 1998; Davis *et al*. 2004). Plant species that are sexually polymorphic are likely to be particularly sensitive to the contingencies of spatial distribution. This is because polymorphic populations are reproductively subdivided into separate sexes (e.g. dioecy), or into distinct mating groups that differ in floral morphology (e.g. heterostyly). As a result, a sexual morph's reproductive success may depend not only on patch density, but also on the local frequency of the sexual morphs with which it can mate (Wyatt & Hellwig 1979; Barrett & Thomson 1982; Meagher 1991; Heilbuth *et al*. 2001; Wilson & Harder 2003). Previous studies have documented frequency-dependent selection in the female fertility of polymorphic species (Eckert *et al*. 1996; McCauley & Brock 1998), as well as density dependence in monomorphic species (Silander 1978; Groom 1998). However, to our knowledge, no study has examined the joint effects of density- and frequency-dependent processes on plant fitness, even though pollinators are known to respond to both the density of flowers and the frequency of floral morphs (Smithson & MacNair 1997).

Here we employ neighbourhood models (Mack & Harper 1977; Weiner 1982; Watkinson *et al*. 1983; Pacala & Silander 1985) to investigate the spatial determinants of mating success in *Narcissus assoanus*, a

sexually polymorphic flowering plant with stigma-height dimorphism. Populations of this species are composed of two floral morphs that differ in style length (Baker *et al*. 2000a,b). In long-styled plants (hereafter L-morph), the stigma is positioned at the same height or above the two stamen levels, whereas in short-styled plants (hereafter S-morph), the stigma is positioned well below the two stamen levels. In *Narcissus* species with stylar polymorphisms, including *N. assoanus*, plants are usually self-incompatible, but fully cross-compatible with other plants in a population regardless of morph identity (Barrett *et al*. 1996). This condition differs from typical heterostylous species, which usually exhibit both self- and intra-morph incompatibility (Barrett & Cruzan 1993). As a result, in *Narcissus* floral morphology has a much stronger influence on governing patterns of outcrossed mating and fertility than in heterostylous plants with self- and intra-morph incompatibility (Barrett & Harder 2005).

Population surveys of *N. assoanus* have revealed wide variation in the relative frequencies of the L- and S-morphs (Baker *et al*. 2000a), which is thought to reflect asymmetries in the mating patterns of the two morphs (Barrett *et al*. 1996; Baker *et al*. 2000b; Cesaro *et al*. 2004). Specifically, the S-morph is likely to be less proficient at intra-morph mating than the L-morph because the large spatial separation of stigmas and anthers (herkogamy) in the S-morph reduces the precision of intra-morph pollen transfer. Manipulative field experiments in *N. assoanus* provide evidence to support this hypothesis as seed set is reduced in patches containing only the S-morph (Thompson *et al*. 2003). Morph-specific differences in mating and fertility imply that the spatial distribution and frequencies of floral morphs within local neighbourhoods are likely to play an important role in determining

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variation in mating success. Previous studies on *Narcissus* have largely dealt with population-level morph ratios (e.g. Baker *et al.* 2000*a*,*b*; Arroyo *et al.* 2002; Barrett *et al.* 2004) and have ignored the reproductive consequences of within-population variation in morph structure.

Here we test two hypotheses that deal with the relation between local floral morph composition and female fertility, and are consequences of morph-specific differences in floral morphology. First, if well developed herkogamy in the S-morph limits intra-morph pollen transfer, we would predict that the fertility of this morph should be insensitive to the number of S-morph neighbours, despite being fully compatible with them. In contrast, the fertility of the S-morph should increase with the number of L-morph neighbours. Second, we predict that because plants of the L-morph can engage in both inter-morph and intra-morph mating, the fertility of this morph should be insensitive to morph identity and fertility will increase similarly with both L- or S-morph neighbours. These hypotheses therefore propose that most mating in the S-morph results from inter-morph pollen transfer (disassortative), whereas mating in the L-morph involves both inter- and intra-morph (assortative) pollen transfer. In essence, mating in the L-morph should be random with respect to the morph composition of local neighbourhoods, whereas mating in the S-morph is likely to be largely disassortative. These hypotheses, while specific to our study system, have broader implications because, if supported, they would demonstrate a level of context-dependence not generally recognized in studies of plant mating.

With restricted seed dispersal and the proposed patterns of mating and fertility, we also predict differences in the local clustering of morphs within populations of *N. assoanus*. Because of the inheritance of style length in *Narcissus* (L-morph *ss*; S-morph *S-*; see Baker *et al.* 2000*b*) and mostly L-biased morph ratios of populations in our study area (Baker *et al.* 2000*a*), plants of the two morphs are likely to segregate different average ratios of the L- and S-morphs. Specifically, high levels of disassortative mating in the S-morph should result in equal frequencies of L- and S-styled plants, whereas due to random mating, the progeny of the L-morph should reflect local morph ratios. We tested this hypothesis by investigating the patterns of style morph clustering within natural populations of *N. assoanus*.

2. MATERIAL AND METHODS

(a) *The study system*
Narcissus assoanus is a diminutive perennial geophyte common in southern France and Spain in lowland garrigues vegetation and upland grassland on limestone. Plants usually produce one yellow flower during March–April with a bowl-shaped corona and a long narrow floral tube. Both floral morphs produce similar amounts of seed following inter-morph and intra-morph cross-pollination. Small amounts of seed are produced upon self-pollination, indicating a leaky self-incompatibility system (Baker *et al.* 2000*b*). Flowers are pollinated primarily by Cleopatra butterflies (*Gonepteryx cleopatra*), hawkmoths (*Macroglossum stellatarum*) and solitary bees (*Anthophora* spp.), and low visitation commonly results in pollen-limitation of seed set (Baker *et al.* 2000*c*). Seeds are shed from dry capsules in May to June and have

no obvious dispersal mechanisms. Because plants are of relatively short stature (10–30 cm), seed shadows are likely to be restricted.

(b) *Study sites and measurements*
We selected eight small lowland populations in a radius of 50 km of Montpellier in southern France in mid-April 2003 during peak flowering (localities are listed in Appendix A). The population sizes used in our study are representative of lowland populations in this region (see Baker *et al.* 2000*a*). In each population, we mapped and recorded the floral morph of all flowering individuals by laying out two perpendicular measuring tapes to record the *X*- and *Y*-coordinates of each plant. We marked each plant with a metal tag to facilitate fruit collection at seed maturation two months later. For each plant we recorded fruit set and seed number per capsule. For plants with two to three flowers (*ca* 7%), we calculated the mean seed set per flower and used this variable for subsequent analyses.

(c) *Statistical methods*
We used a neighbourhood model to analyse the relation between seed set and the local density of L- and S-plants. Below we describe the model and our analysis in four steps. First, we describe the structure of the data and the zero-inflated Poisson (ZIP) distribution used to specify the distribution of errors. Second, we explain how the neighbourhood model was used to regress seed set against the local density of plants of the L- and S-morph. Third, we describe how we tested the two hypotheses outlined in §1. Finally, we describe the methods that we used to examine the local clustering of morphs.

Zero-inflated count data. Count data are often analysed using standard Poisson regression methods, based on the assumption that the data exhibits a unimodal distribution. However, visual inspection of our data revealed that seed set was not unimodal because approximately 20% of plants failed to set seed. Therefore, we used a ZIP distribution (Lambert 1992; Heilbron 1994) to account for the moderately high incidence of reproductive failure. The ZIP distribution accommodates zero-inflation by specifying the probability of zero and non-zero observations separately

$$P(Y = 0) = p + (1-p)e^{-\lambda}, \tag{2.1a}$$

$$P(Y = y) = (1-p)\frac{e^{-\lambda}\lambda^y}{y!}, \quad y > 0, \tag{2.1b}$$

where y is the observed seed set per flower, λ is the mean of the Poisson distribution, p is the probability of observing a surplus of zeros (above and beyond that expected from the Poisson distribution) and the mean seed set ($\bar{y}$) is equal to $(1-p)\lambda$.

Regressing seed set against local density. In standard Poisson regression, only the mean of the Poisson distribution, λ, varies as function of covariates. In a ZIP regression, however, both λ and p may vary as a function of covariates. Thus, for the L-morph, we estimated λ and p as linear functions of local density

$$p_l = \alpha + \beta N_s + \phi N_l, \tag{2.2a}$$

$$\lambda_l = \sigma + \theta N_s + \varphi N_l, \tag{2.2b}$$

where N_l and N_s are the number of plants of the L- and S-morph within a distance R of the focal plant. For the S-morph, we assumed that pollen transfer was largely

disassortative (this assumption will be evaluated later), and estimated λ and p as linear functions of N_1 alone

$$p_s = \alpha + \phi N_1, \tag{2.3a}$$

$$\lambda_s = \sigma + \varphi N_1. \tag{2.3b}$$

It is reasonable to assume that seed set will vary with the local density of plants because pollen supply has previously been shown to limit seed set (Baker *et al.* 2000*c*; Thompson *et al.* 2003). However, it is not known how far pollen is dispersed by insects, so it was difficult to determine *a priori* how large R should be for calculation of N_1 and N_s. Nevertheless, the scale at which neighbours influence seed set can be inferred from the data itself (Pacala & Silander 1985). For example, it is possible to calculate N_1 and N_s using a range of different values for R (e.g. 1 m to the maximum distances in populations), and then determine which value of R provides the best fit to the observed seed set. Thus, R becomes a parameter, hereafter called neighbourhood radius that we estimated along with the other parameters listed above.

To estimate the parameters, we used a simulating annealing algorithm to search for parameter values that maximized the log-likelihood of the data, thereby obtaining a maximum likelihood estimate for each of the parameters included in equations (2.2*a*,*b*) and (2.3*a*,*b*) (α, β, ϕ, σ, θ, φ and R). We also calculated confidence intervals for each parameter as follows. First, we generated 10 000 random values for each of the model parameters, and combined them to obtain 10 000 unique sets of the model parameters. Then, we calculated the log-likelihood of each parameter set, and its deviance from the maximum log-likelihood ($2(L-L_{max})$), and excluded those sets for which the deviance exceeded the critical value of the χ^2 distribution ($\alpha = 0.05$, d.f. $= 1$). Finally, we selected the minimum and maximum parameter values from the remaining sets and used these as the 95% confidence limits.

Hypothesis tests. The regression models outlined above (equations (2.2*a*,*b*) and (2.3*a*,*b*)) embody two assumptions that correspond to the first two hypotheses outlined in §1. First, pollen transfer in the S-morph is assumed to be disassortative, because λ_s and p_s are allowed to covary with N_1 but not N_s (equations (2.3*a*,*b*)). Second, pollen transfer in the L-morph is assumed to involve both assortative and disassortative components, because λ_1 and p_1 are allowed to covary with N_s as well as N_1 (equations (2.2*a*,*b*)).

To test these two assumptions, and the related hypotheses, we formulated two alternative regression models and compared them to our null models (equations (2.2*a*,*b*) and (2.3*a*,*b*)) using likelihood ratio tests. The first alternative model allows λ_s and p_s to covary with N_s as well as N_1, as would be expected if the S-morph were capable of significant assortative pollen transfer

$$p_s = \alpha + \pi N_s + \phi N_1, \tag{2.4a}$$

$$\lambda_s = \sigma + \tau N_s + \varphi N_1. \tag{2.4b}$$

The second alternative model allows λ_1 and p_1 to covary with N_1 but not N_s, as would be expected if L-morphs were not capable of assortative pollen transfer

$$p_1 = \alpha + \phi N_1, \tag{2.5a}$$

$$\lambda_1 = \sigma + \varphi N_1. \tag{2.5b}$$

The maximum log-likelihood of these alternative models was computed as described previously, then subtracted from the maximum log-likelihood of the full model (equations

(2.2*a*,*b*) and (2.3*a*,*b*)). The alternative model was deemed to fit the data better than the null model if the difference in the log-likelihood exceeded twice the critical value of the χ^2 distribution ($\alpha = 0.05$, d.f. $= q$, where q is the difference in the number of model parameters).

We also predicted that pollen transfer in the L-morph was random with respect to morph (i.e. seed set increases to an equal degree with the local density of plants of the L- and S-morphs). An assumption of equal slopes was not built into the null model (equations (2.2*a*,*b*)) because preliminary analyses showed that, among plants that set seed, the number of seeds (λ_1) increased faster with the local density of the L-morph than it did with the local density of the S-morph (i.e. $\theta > \varphi$). However, it was also noted that the probability of observing surplus zeros (p_1) decreased with the local density of the S-morph (i.e. $\beta < 0$), thereby compensating for the fact λ_1 increased faster with respect to N_1 than N_s. In other words, the function used to specify total seed set ($\bar{y}_1 = (1-p_1)\lambda_1$) increases approximately equally with both N_1 and N_s, even though λ_1 and p_1 do not.

Unfortunately, it was not possible to conduct a formal test of the equal slopes hypothesis because $\bar{y}_1$ is a compound function of λ_1 and p_1, and is therefore slightly nonlinear with respect to both N_1 and N_s (i.e. there is no single slope with respect to N_1 or N_s). However, we plotted the fitted function in three dimensions ($\bar{y}_1$ versus N_1 and N_s) to visually evaluate whether the slopes are approximately equal (see §3). We also calculated the average slope of $\bar{y}_1$ with respect to N_1 and N_s at equally spaced locations ($i = 1, ..., 25$) on the N_1–N_s plane

$$\overline{\partial \bar{y}_1/\partial N_1} = \frac{\sum\limits_{i=1}^{25} \partial \bar{y}_1/\partial N_1}{25}, \tag{2.6a}$$

$$\overline{\partial \bar{y}_1/\partial N_s} = \frac{\sum\limits_{i=1}^{25} \partial \bar{y}_1/\partial N_s}{25}. \tag{2.6b}$$

The null models (equations (2.2*a*,*b*) and (2.3*a*,*b*)) embody an additional assumption that we did not discuss above, i.e. that seed set of both morphs increases to an equal degree with the local density of the L-morph (because α, β, σ and θ are common to both equations). To evaluate this assumption, we formulated alternative models that allow λ_s and p_s to differ from λ_1 and p_1 even in the absence of S-morph neighbours

$$p_s = \varepsilon + \rho N_1, \tag{2.7a}$$

$$\lambda_s = \upsilon + \omega N_1. \tag{2.7b}$$

These models were compared to the null models using likelihood ratio tests as described previously.

Clustering of morphs. The clustering of morphs is likely to influence seed set if both pollen and seed dispersal are local. To test for clustering, we calculated the average difference between the population and neighbourhood morph ratios as follows

$$d_1 = \frac{\sum\limits_{j=1}^{8} \sum\limits_{i=1}^{n_1} \left(\frac{n_1}{(n_s + n_1)} - \frac{N_1}{(N_s + N_1)} \right)}{\sum\limits_{j=1}^{8} n_1}, \tag{2.8a}$$

$$d_s = \frac{\sum\limits_{j=1}^{8} \sum\limits_{i=1}^{n_s} \left(\frac{n_1}{(n_s + n_1)} - \frac{N_1}{(N_s + N_1)} \right)}{\sum\limits_{j=1}^{8} n_s}, \tag{2.8b}$$

Table 1. Sample sizes and means for the measurements of plants from eight populations of *Narcissus assoanus* studied in southwestern France. (Number of individuals per floral morph (N L, N S), number of total individuals, number of flowers, the morph ratios (number of plants of the L-morph divided by the total number of individuals) and the mean seed set per flower for each morph.)

pop.	N L	N S	N total	N flowers	morph ratio	seeds L (s.e.)	seeds S (s.e.)
1	46	55	101	102	0.46	14.0 (2.1)	11.7 (1.5)
2	77	46	123	127	0.63	8.5 (1.3)	6.8 (1.6)
3	19	50	69	69	0.28	14.8 (2.2)	9.5 (1.8)
4	91	22	113	113	0.81	19.1 (1.7)	13.4 (3.0)
5	41	30	71	77	0.58	10.6 (1.5)	14.0 (2.1)
6	54	35	89	96	0.61	13.5 (1.6)	13.2 (1.7)
7	39	50	89	97	0.44	17.6 (2.6)	11.8 (1.4)
8	38	42	80	80	0.48	13.0 (2.5)	6.5 (1.9)
total	405	330	735	761	0.55	13.9 (0.7)	10.9 (0.6)
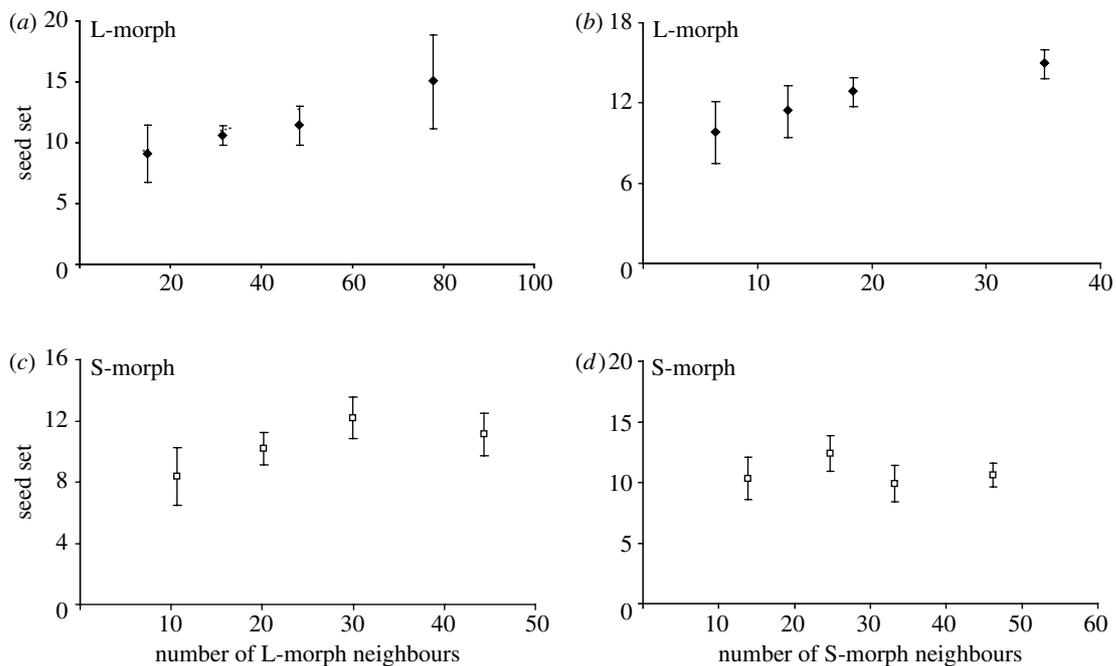
Figure 1. The mean seed set of (*a*, *b*) the L-morph and (*c*, *d*) S-morph in eight populations of *N. assoanus* in relation to the number of plants of the L- and S-morph in a neighbourhood radius R of 10.04 m. We calculated mean values by dividing the number of respective neighbours into four bins (i.e. $N_s < 20$, $20 \leq N_s < 30$, $30 \leq N_s < 40$, $N_s \geq 40$), then calculating the mean seed set ($\pm$s.e.) and the mean number of neighbours for all observations falling within each bin. The width of the bins and their location on the X-axis vary along the axes (and between panels) because they were chosen to illustrate positive relationships between seed set and neighbourhood density wherever they existed.

where N_l and N_s are calculated using the maximum-likelihood estimate of R (same as equations (2.8*a*,*b*) above). Finally, to enable comparison, we also computed these same mean differences for a whole range of neighbourhood radii (1–30 m).

3. RESULTS

(a) *General patterns*

Our eight study populations were composed of 69–124 flowering individuals (mean 92.0). Morph ratios varied from L-biased (0.81) to S-biased (0.28), with a mean morph ratio of 0.55 (table 1). Fruits were obtained from 64.9% of the original sample of flowers. There were significant differences in fertility between style morphs (Appendix B; Mann–Whitney U-test, $\chi^2 = 4.75$,

$p = 0.029$). The L-morph produced on average more seeds per capsule (mean 13.9, $\pm$s.e. $= 0.7$) than the S-morph (10.9, $\pm$s.e. $= 0.6$) in seven out of eight populations (table 1). There was no consistent relation between mean seed set per morph and population morph ratio, or between seed set per morph and population size (Spearman rank test, all p-values $\gg 0.05$).

(b) *Local morph structure and female fertility*

As predicted, the seed set of floral morphs in *N. assoanus* responded differently to local morph structure. Observed fertility of the L-morph increased with the number of neighbouring plants of both the L- and S-morphs (figure 1*a*,*b*) implying that pollen transfer to the L-morph involves both assortative and disassortative pollination. The likelihood ratio test confirmed that the null model
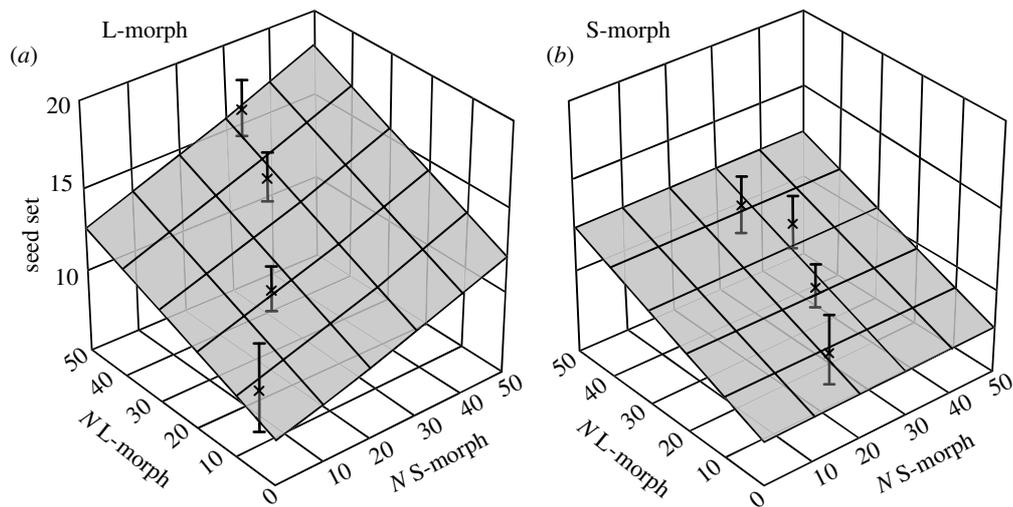
Figure 2. The fitted relation between morph-specific seed set and the number of neighbours in eight populations of *Narcissus assoanus*. (*a*) The fitted relation between the seed set of the L-morph and the number of neighbours (equations (2.3*a*,*b*)). Data points indicate the mean number of seeds and the mean number of neighbours within each of four bins. We calculated mean values by summing the number of L- and S-styled neighbours ($N_l + N_s = N_l$), then dividing the data into four bins based on the total number of neighbours (i.e. $N_t < 30$, $30 \leq N_t < 50$, $50 \leq N_t < 70$, $N_t \geq 70$), and calculating the mean seed set ($\pm$s.e.), and the mean number of neighbours (N_l and N_s) for all observations falling within each N_t bin. For computational details refer to the text. (*b*) The fitted relation between the seed set of the S-morph and the number of neighbours (equations (2.3*a*,*b*)), with data points indicating the mean number of seeds and the mean number of neighbours within each of four bins. We calculated mean values by dividing the N_l-axis into four bins, then calculating the mean seed set ($\pm$s.e.) and the mean number of neighbours (N_l and N_s) for all observations falling within each N_l bin. The width of the bins vary along the N_l-axis because they were chosen to illustrate positive relationships between seed set and the number L-morph neighbours.

(equations (2.2*a*,*b*); assortative and disassortative pollen transfer) fitted the data significantly better ($p < 0.05$, d.f. $= 2$) than the alternative model (equations (2.5*a*,*b*); only disassortative pollen transfer). Indeed, the two slopes of the null model (slopes along the N_l and N_s axes) appear to be similar to one another (figure 2*a*), suggesting that both floral morphs are equally proficient at pollinating plants of the L-morph resulting in random pollination. This visual interpretation is consistent with the similarity of the calculated values of the mean slope (equations (2.6*a*,*b*)) of $\bar{y}_l$ with respect to N_l (0.107) and N_s (0.093).

The observed seed set of the S-morph increased with the number of neighbouring L-morphs (figure 1*c*), but was insensitive to the number of neighbouring S-morphs (figure 1*d*). This implies that most pollen transfer to the S-morph was disassortative (figure 1*c*). This was corroborated by the likelihood ratio test: the alternative model allowing both assortative and disassortative pollen transfer (equations (2.4*a*,*b*)) did not fit the data significantly better ($p > 0.05$, d.f. $= 2$) than the null model (equations (2.3*a*,*b*); strictly disassortative pollen transfer). Thus, the null model was the most parsimonious model for explaining fertility relations in the S-morph (figure 2*b*).

The final likelihood ratio test also confirmed that seed set of the two morphs increased to an equal degree with the number of neighbouring L-morphs. In particular, the alternative model (equations (2.8*a*,*b*), in which λ_s and p_s are allowed to differ from λ_l and p_l) did not provide a significantly better fit ($p > 0.05$, d.f. $= 4$) than the null model (equations (2.3*a*,*b*)).

(**c**) *Average neighbourhood size*

The value of R that provided the best fit to the data (i.e. the maximum likelihood estimate) was 10.04 m (Appendix B). In contrast, the area of the largest and smallest populations measured were 12×15.5 m and $17.5 \times$

34 m, respectively. The difference implies that not all plants in a population influence the seed set of focal individuals. Indeed, the mean number of L- and S-morph neighbours ($\bar{N}_l$ and $\bar{N}_s$) within the distance R were 31.5 and 26.5, respectively, much smaller than the size of most populations (table 1). Thus, even within these relatively small populations, local mating within restricted neighbourhoods seems likely.

(**d**) *Spatial clustering of morphs*

As predicted from the inferred mating patterns in *N. assoanus*, the two morphs differed in their representation in local neighbourhoods of each morph. Average morph ratios in the vicinity of plants of the S-morph were less L-biased than the average population morph ratio, particularly at distances less than 5 m (figure 3). In contrast, average morph ratios in the vicinity of plants of the L-morph were more L-biased than the average population morph ratio, again especially at distances less than 5 m.

4. DISCUSSION

Morph ratios in sexually polymorphic plants are a visible signature of the mating patterns that have occurred in populations during preceding generations. The distributions of morphs within a population also provide the spatial template for subsequent mating patterns since pollen dispersal is usually local, particularly in animal-pollinated species. In *N. assoanus*, we have demonstrated that the floral morph composition of local neighbourhoods has an important influence on variation in female fertility. By inferring expected patterns of pollen transfer based on floral morphology, we predicted the relations between local morph ratios and patterns of seed set. Below we discuss the likely mechanisms responsible for our results and also highlight the strong context-dependence of
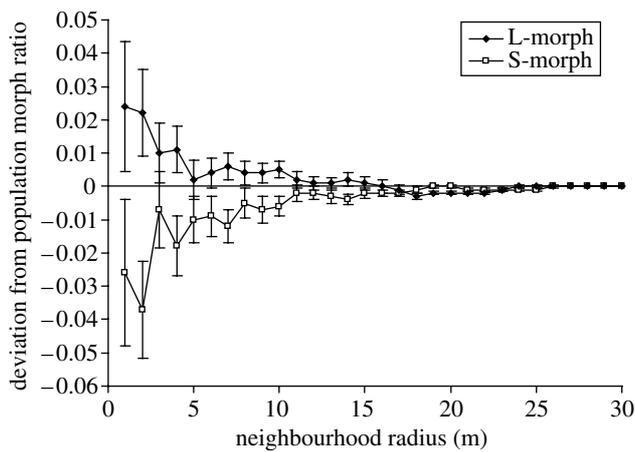
Figure 3. The mean difference between the population morph ratio and the morph ratio of plants surrounding individuals of the L- and S-morph in eight populations of *Narcissus assoanus* calculated for each of 30 different neighbourhood radii (1–30 m; equations (2.8*a*,*b*)). Deviations above zero indicate that the local morph ratio is more L-biased than the population morph ratio, whereas deviations below zero indicate that the local morph ratio is less L-biased than the population morph ratio. For computational details refer to the text.

mating opportunities in structured plant populations that arise from density- and frequency-dependent processes. We argue that such context-dependence may be a general feature of the ecology of plant mating.

(a) *Morphology, mating and morph ratios*

In distylous plants, heteromorphic self-incompatibility prevents intra-morph mating and most of the seed produced in a population results from inter-morph mating. Because of the simple Mendelian inheritance of distyly, maternal parents segregate roughly equal numbers of L- and S-morphs. Provided that sexual recruitment predominates, and there are no fitness differences between the floral morphs, morph ratios should be close to unity at both the population and neighbourhood levels (but see Levin 1975). Any deviations in morph ratios at a local scale will usually result from sampling variance. Our studies of morph ratios within and between populations of *N. assoanus* depart significantly from this situation.

Symmetrical disassortative mating and 1 : 1 morph ratios occur rarely in *Narcissus* because floral morphology rather than physiological mechanisms governs mating patterns (Barrett & Harder 2005). Imperfect sex-organ reciprocity and the associated differences in herkogamy between the floral morphs have been proposed as a major cause of asymmetrical mating patterns and biased morph ratios (Barrett *et al*. 1996; Baker *et al*. 2000*a*,*b*). This explanation assumes that, unlike typical heterostylous species, the floral morphs in *Narcissus* are not functionally equivalent as mating partners. This hypothesis was supported by our results. There were significant differences between the floral morphs in their reproductive behaviour, depending on the morph identity of neighbours (figure 2). Pollen transfer and mating in *N. assoanus* is, therefore, context-dependent because of the spatial heterogeneity of morph frequencies at a local scale. This spatial heterogeneity is further reinforced because asymmetrical mating results in different patterns of segregation in the floral morphs.

The fertility of L-styled plants increased significantly with the total number of plants in local neighbourhoods. This pattern occurred regardless of morph identity implying that the L- and S-morph were functionally equivalent as paternal mating partners (figures 1*a*,*b* and 2*a*). In the L-morph, density-dependent rather than frequency-dependent processes play a more significant role in governing fertility variation. In contrast, the fertility of the S-morph was influenced by the morph identity of plants in local neighbourhoods. Increased fertility was positively associated with the number of neighbouring L-styled plants, whereas the number of S-styled plants had no influence on variation in seed set (figures 1*c*,*d* and 2*b*). Hence, both density- and frequency-dependent control of fertility variation was evident in the S-morph. This difference in mating behaviour of the floral morphs may be associated with their contrasting evolutionary histories and numerical occurrence in contemporary populations (reviewed in Barrett & Harder 2005).

What specific features of sex-organ deployment promote asymmetrical mating in *N. assoanus*? Both floral morphs possess upper-level anthers positioned for pollen donation to stigmas of the L-morph, but lower-level anthers differ consistently in position (see fig. 1 in Baker *et al*. 2000*a*). In the S-morph, lower-level anthers are positioned just below upper-level anthers and both stamen levels probably function primarily in pollen donation to stigmas of the L-morph. In contrast, lower-level anthers of the L-morph are positioned further down the floral tube and probably provide most of the pollen that fertilizes ovules of S-styled plants. Because of these differences in lower stamen position, the S-morph is probably mostly engaged in inter-morph mating, whereas stamen differentiation in the L-morph promotes both intra- (via upper-level anthers) and inter-morph (via lower-level anthers) mating. The morph-specific differences in the relations between local morph ratios and seed set that we observed in our study support these inferences on the influences of stamen position on pollen transfer. Moreover, phenotypic models of pollen transfer (Barrett *et al*. 1996) and genetic mating models (Baker *et al*. 2000*b*) that take into account these specific details of morphology in *Narcissus* demonstrate that asymmetrical mating can result in the patterns of morph-ratio variation that is observed in *N. assoanus*.

We also observed differences in the clustering of *N. assoanus* morphs that is consistent with inferred morph-specific patterns of mating and restricted seed dispersal (Hamrick & Nason 1996; Stehlik & Holderegger 2000; Kalisz *et al*. 2004). On the scale of less than 5 m, average morph ratios in the vicinity of S-morph individuals were less L-biased than the mean population morph ratio, whereas average morph ratios in the vicinity of L-morph individuals were more L-biased than the average population morph ratio (figure 3). This result suggests that the difference in spatial clustering may have contributed to the observed average lower fertility of the S-morph (table 1). However, deviations from the average population morph ratio were small, with maximum differences of less than 5% for both morphs (figure 3). Furthermore, at a scale of 10 m (the scale we have estimated to be most relevant to seed set), neighbourhood morph ratios were not discernibly different than population morph ratios. This result implies that pollen dispersal distances in *N. assoanus* exceed the size of the clusters created by local seed dispersal.

(b) General implications

Our study demonstrates the context-dependent nature of plant mating (see Kohn & Barrett 1994; Kalisz *et al*. 2004) with both density- and frequency-dependent processes influencing the reproductive success of floral morphs in *N. assoanus*. Previous studies of plant populations have revealed both density-dependent influences on female fertility variation (Silander 1978; Groom 1998) and frequency-dependent selection in polymorphic species (Eckert *et al*. 1996; McCauley & Brock 1998). However, to our knowledge our study is the first to reveal their joint effects on plant fitness, although both processes are known to influence pollinators such as bumble-bees (Smithson & MacNair 1997). Populations of animal-pollinated plants commonly maintain considerable phenotypic diversity in floral traits involving both polymorphic and quantitative variation. If pollinators respond to this variation in a frequency- or density-dependent manner, such behaviour could have important consequences for selection on floral traits (Smithson 2001).

We have used neighbourhood models to examine the relation between female fertility and the local morph composition of neighbourhoods. These models enabled us to test several hypotheses concerning the patterns of pollen transfer and mating in populations of *N. assoanus*. Although similar methods have been employed to infer spatial patterns of plant competition (Mack & Harper 1977; Weiner 1982; Watkinson *et al*. 1983; Pacala & Silander 1985), this is their first application to the study of mating patterns in plant populations. Measurements of pollen dispersal and patterns of assortative and disassortative mating are technically demanding since they require pollen markers and hypervariable genetic loci, respectively, that are not available for most plant species. The modelling framework used here in combination with manipulative field experiments (e.g. Thompson *et al*. 2003) can provide an alternative approach for determining the complex ecological and genetic mechanisms that govern mating success in plants.

APPENDIX A. Coordinates of the eight populations of *Narcissus assoanus* studied in southwestern France

pop.	coordinates N/E
1	43.9685/3.9804
2	43.8343/3.8987
3	43.9444/3.7477
4	43.6863/3.8265
5	43.6863/3.8265
6	43.7904/3.8539
7	43.9555/3.8190
8	43.9444/3.7543

APPENDIX B. Maximum likelihood estimates (with confidence limits) of α, β, ϕ, σ, θ, φ and R used to predict seed set (equations (2.2*a,b*) and (2.3*a,b*)) of the L- and S-morph in eight populations of *Narcissus assoanus*

parameter	estimated value	lower confidence limit	upper confidence limit
α	0.26	0.22	0.29
β	-0.0032	-0.0043	-0.0012
ϕ	0.0	-0.0015	0.0012
σ	10.43	9.88	11.28
θ	0.06	0.04	0.08
φ	0.13	0.12	0.15
R	10.04	9.74	10.57

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