HAL open science

Variation of a floral polymorphism at different spatial scales in the Mediterranean geophyte Narcissus assoanus

Regina Berjano, Perrine Gauthier, Chiara Parisi, Valentine Vaudey, Virginie Pons, Alain Renaux, David Doblas, John D. Thompson

To cite this version:

Regina Berjano, Perrine Gauthier, Chiara Parisi, Valentine Vaudey, Virginie Pons, et al.. Variation of a floral polymorphism at different spatial scales in the Mediterranean geophyte Narcissus assoanus. Journal of Plant Ecology, 2016, 9 (3), pp.333-345. ⟨10.1093/jpe/rtv057⟩. ⟨hal-04852090⟩

HAL Id: hal-04852090

https://hal.science/hal-04852090v1

Submitted on 1 Jan 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons CC BY 4.0 - Attribution - International License

Variation of a floral polymorphism at different spatial scales in the Mediterranean geophyte *Narcissus assoanus*

Regina Berjano[1,2,], Perrine Gauthier[1], Chiara Parisi[1], Valentine Vaudey[1], Virginie Pons[1], Alain Renaux[1], David Doblas[1] and John D. Thompson[1]*

[1] *UMR 5175 Centre d'Ecologie Fonctionnelle et Evolutive, CNRS, 1919 route de Mende, 34293 Montpellier cedex 5, France*
[2] *Departamento de Biología Vegetal y Ecología, University of Seville, Apdo 1095. 41080 Seville, Spain*
*Correspondence address. R. Berjano, Departamento de Biología Vegetal y Ecología, University of Seville. Apdo 1095. 41080 Seville, Spain. Tel: +34-954552763; Fax: +34-954557059; E-mail: regina@us.es

Abstract

Aims
Habitat fragmentation impacts the spatial extent and isolation of local populations and communities. Although the biological consequences of these impacts have been well studied at the site level, effects directly related to changes in the spatial configuration of populations in the landscape remain less clear. The objective of this study is to examine how changes in the spatial-scale configuration of populations are associated with variability in morph rations in the floral polymorphic Mediterranean geophyte *Narcissus assoanus*.

Methods
We performed a nested analysis of morph ratio variation at three spatial scales: a 50 × 50 km regional scale in SE France, in fourteen 1 × 1 km landscapes located in the same region, and within 12 spatially extensive population patches. We also quantified variation in the behaviour of pollinators in population patches of contrasting spatial configuration.

Important Findings
At a regional scale, morph ratios show a geographic pattern of increasing isoplethy (1:1 morph ratio) away from centres of human population development and in upland pastures. At the landscape scale, isoplethy of local population patches is more correlated with decreased isolation among patches than with patch size. Finally, within local isoplethic populations, small patches can show significantly biased morph ratios. In small isolated patches, pollinators perform shorter flight distances among consecutive flower visits than in spatially extensive patches. This study of variation in a genetic polymorphism at multiple spatial scales provides new insights into the scale-dependent effects of habitat fragmentation and the potential occurrence of metapopulation dynamics in natural plant populations.

Keywords: flower polymorphism, habitat fragmentation, Mediterranean, Narcissus, stigma-height polymorphism

INTRODUCTION

The fragmentation of natural habitats by increasing human land use and development has become a major driver of changes in biodiversity (Sala *et al.* 2000). Habitat fragmentation can cause both a spatial reduction of natural habitats with a decline in numbers of individuals or species and isolation among patches of habitat that disrupts gene flow (Fahrig 2003; Thomas *et al.* 2001; Wilcove *et al.* 1986). Although the major effect of fragmentation on biodiversity may stem from the loss of available habitat (Fahrig 2003), effects due to the spatial configuration of habitats (i.e. the pattern and connectivity of different elements in a landscape), although less studied, can also affect community composition and population dynamics (Chetkiewicz *et al.* 2006; Hilty *et al.* 2006; Öckinger *et al.* 2011; Taylor *et al.* 1993).

In plants, fragmentation causes changes in species composition (Arroyo-Rodríguez *et al.* 2009; Damschen *et al.* 2008;

Lindborg and Eriksson 2004) and the spatial organization of genetic diversity (Young *et al.* 1996), and affects sexual reproduction (Aizen *et al.* 2002; Ashworth *et al.* 2004; Aguilar *et al.* 2006; Ward and Johnson 2005; Wagenius 2006; Winter *et al.* 2008). Also, as a result of impacts on insect communities, insect pollinators may decline (Cozzi *et al.* 2008; Goverde *et al.* 2002; Krauss *et al.* 2003a; Öckinger and Smith 2006; Sowig 1989; Steffan-Dewenter and Tscharntke 1999, 2000, 2002) and show changes in behaviour (Bernhardt *et al.* 2008; Goverde *et al.* 2002). In particular, interplant flight distances of pollinators may depend on the spatial organization of plants and their flowers (Cartar and Real 1997; Cresswell 2000; Dauber *et al.* 2010).

Habitat fragmentation can also affect floral polymorphisms (populations with two or more floral morphs) in species with floral morphs that are differentially affected by habitat fragmentation and its impact on pollination (Barrett *et al.* 1989; Husband and Barrett 1992b; Matsumura and Whashitani 2000; Van Rossum *et al.* 2006). In addition, morph ratio variation may be linked to the dynamics of colonization if the genetic assemblage of colonizing populations randomly causes a decline in the abundance or loss of particular morphs (Barrett *et al.* 1989; Eckert and Barrett 1992; Endels *et al.* 2002; Husband and Barrett 1992a; Hodgins and Barrett 2008; Kéry *et al.* 2003). Such changes in morph frequency illustrates how fragmentation may induce changes in evolutionary trajectories (Lindenmayer and Fisher 2007).

In the Mediterranean geophyte *Narcissus assoanus*, populations contain two floral morphs and show either isoplethic (i.e. statistically similar to 1:1) or long-styled morph (L-morph) biased ratios (Baker *et al.* 2000b; Thompson 2005; Thompson *et al.* 2012). The precise causes of this variation are unclear. Baker *et al.* (2000b) reported that morph ratios become L-morph biased as a result of reduced population size (based on an estimate of plant numbers). However they did not examine the contribution of the spatial configuration of populations in the landscape or variation in pollinator composition. In other *Narcissus* species with a stigma-height polymorphism, morph ratio variation has indeed been found to be associated with changes in pollinators in different parts of their range (Arroyo *et al.* 2002; Pérez-Barrales and Arroyo 2010; Santos-Gally *et al.* 2013).

The overall aim of this study is to assess the relative contribution of the different elements of spatial configuration of populations in the landscape to variation in morph ratio among populations of *N. assoanus*. To do so, we analysed morph ratio variation at three spatial scales. First, to confirm the overall pattern of morph ratio variation at the scale studied in previous work, populations were extensively sampled on a 'regional scale' of 50 × 50 km, which encompasses an ecological and climatic transition from lowland garrigues to upland rocky pastures. Second, to test whether the spatial configuration of populations impacts morph ratio variation over and above any effect of population size (detected by Baker *et al.* 2000b), morph ratio variation was quantified in a replicated 'landscape scale' study of all populations patches present within zones of 1 km^2. Third, morph ratios were quantified at a 'within-population-scale' in order to assess whether the fragmentation of an existing population could produce biased morph ratios by chance. Finally, to assess a potential contribution of pollinator behaviour to morph ratio variation we performed observations of pollinator visitation, and to remain comparative with previous studies we quantified female reproductive success in different population contexts.

METHODS

Study species

Narcissus assoanus (Amaryllidaceae) is a small geophyte, 10–15 cm tall, widespread on limestone in Mediterranean France and Spain. In Mediterranean France, it typically occurs in open garrigues and stony pastures from sea level to 900 m elevation. Individual bulbs produce a single-stemmed inflorescence with most often one, occasionally two or very rarely three deep-yellow flowers, which flower from March to early May depending on elevation. Flowers possess two stamen levels each with three anthers and a marked stigma-height dimorphism (Baker *et al.* 2000b; Thompson *et al.* 2012). Long-styled flowers (L-morph) have a stigma that occurs close to or above the anthers, and short-styled flowers (S-morph) have a stigma positioned well below the anthers in the floral tube (Fig. 1). *N. assoanus* is partially self-sterile but does not exhibit heteromorphic incompatibility (Baker *et al.* 2000c).

Regional scale

We quantified morph ratios on a 'regional scale' in a total of 144 populations (see online supplementary Appendix S1) in a 50 × 50 km area located to the north and west of Montpellier in southern France (Fig. 2). In this area, previous samples of populations at this scale have shown that morph ratio varies from isoplethy in large continuous populations in upland

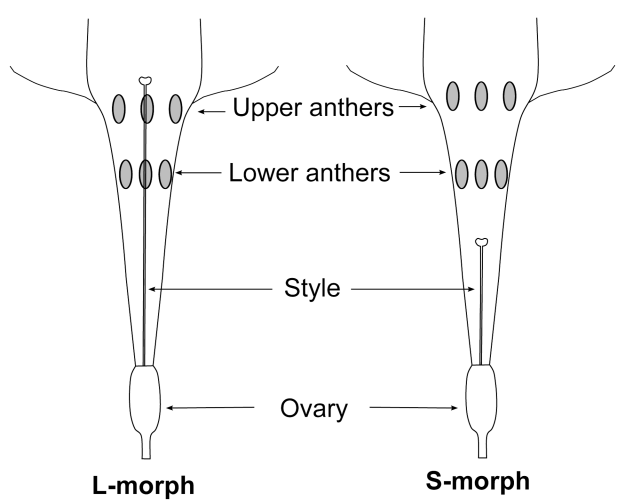

Figure 1: schematic representation of the two floral morphs in *N. assoanus*.

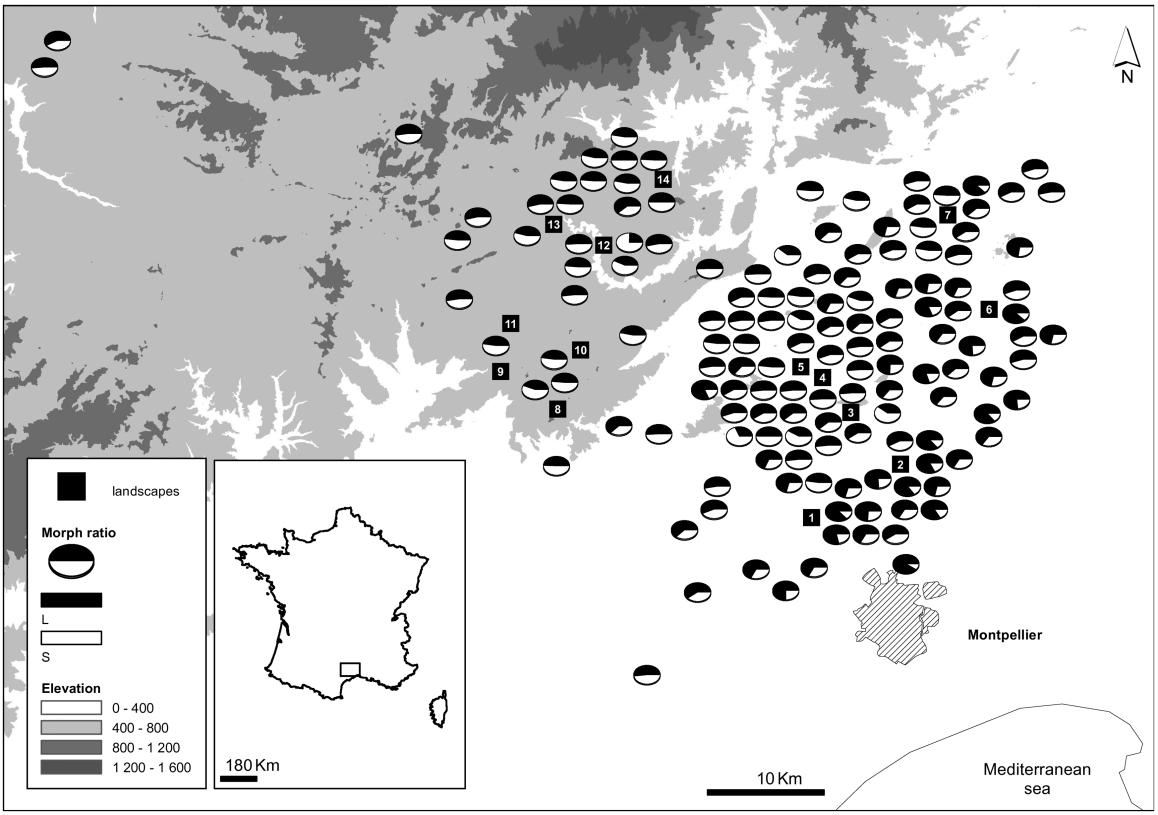

Figure 2: location of the study area (squared) and spatial distribution of morph ratios (proportion of the L-morph in black, S-morph in white) in 144 populations of *Narcissus assoanus* in southern France. The number in the black squares refers to the fourteen zones used for the landscape-scale study.

sites to strongly L-morph biased ratios in lowland sites (Baker *et al.* 2000b; Thompson 2005). Populations in lowland sites occur in garrigues and rocky pastures at < 500 m elevation, and are often highly fragmented by human activities (agriculture, roads, urban development), while populations to the north-west occur on upland limestone plateaux at 500–900 m elevation in more continuous, open extensively grazed rocky pastures. Georeferences for all populations were obtained with a Global Positioning System (GPS) navigation device (Garmin® XTRAIL; error < 10 m). To quantify morph ratios, flowers were sampled randomly by walking through a population and sampling flowers at least 0.5 m apart to avoid resampling the same individual (bulbs can fragment, so an individual genotype can have several flowering stems). In order to check for any short-term variability, the morph ratio of 14 populations assessed in 1998 was repeated in the period between 2001 and 2006.

Landscape scale

Across the regional study area, we selected 14 zones of presence of *N. assoanus* each one in an area of 1 km^2 to provide the basis for an analysis of morph ratio variation at the 'landscape scale' (Fig. 3). For most of these zones, we were able to study a 1 × 1 km square, but in some of them it was not possible to have a square due to marked natural discontinuities (dense forest, steep outcrops or inaccessible areas). Each 1 km^2 zone was visited on several occasions to map the occurrence of the species.

Populations were frequently delimited by natural and artificial discontinuities of the habitat occupied by the study species in the fourteen zones. These discontinuities were both natural, due to watercourses (mostly temporal), rocky escarpments and screes, woodland and dense scrubland typical of the study region, and the result of human activities—vineyards and other cultivation, large farms, gardens and small hamlets, roads and pipelines. However, very short distances separated some population patches as a result of the presence of fairly narrow discontinuities in the open garrigues habitat of the study species. Hence, in the study of spatial configuration of populations we use the term 'patch' or 'population patch' instead of population. Several patches of suitable habitat were explored and mapped to the limits of occurrence of plants but contained only very low densities of plants and not enough flowers to do a correct morph ratio (<50 flowers). These areas are included in the total occupied surface area and used as nearest neighbours in the analysis of distance for patches with a morph ratio. Hence, in each zone there can be population patches with no morph ratio. We also mapped patches beyond the boundaries of the zone to be sure that we correctly assess the distance to the closest patch for all patches within the 1 km^2 zone.

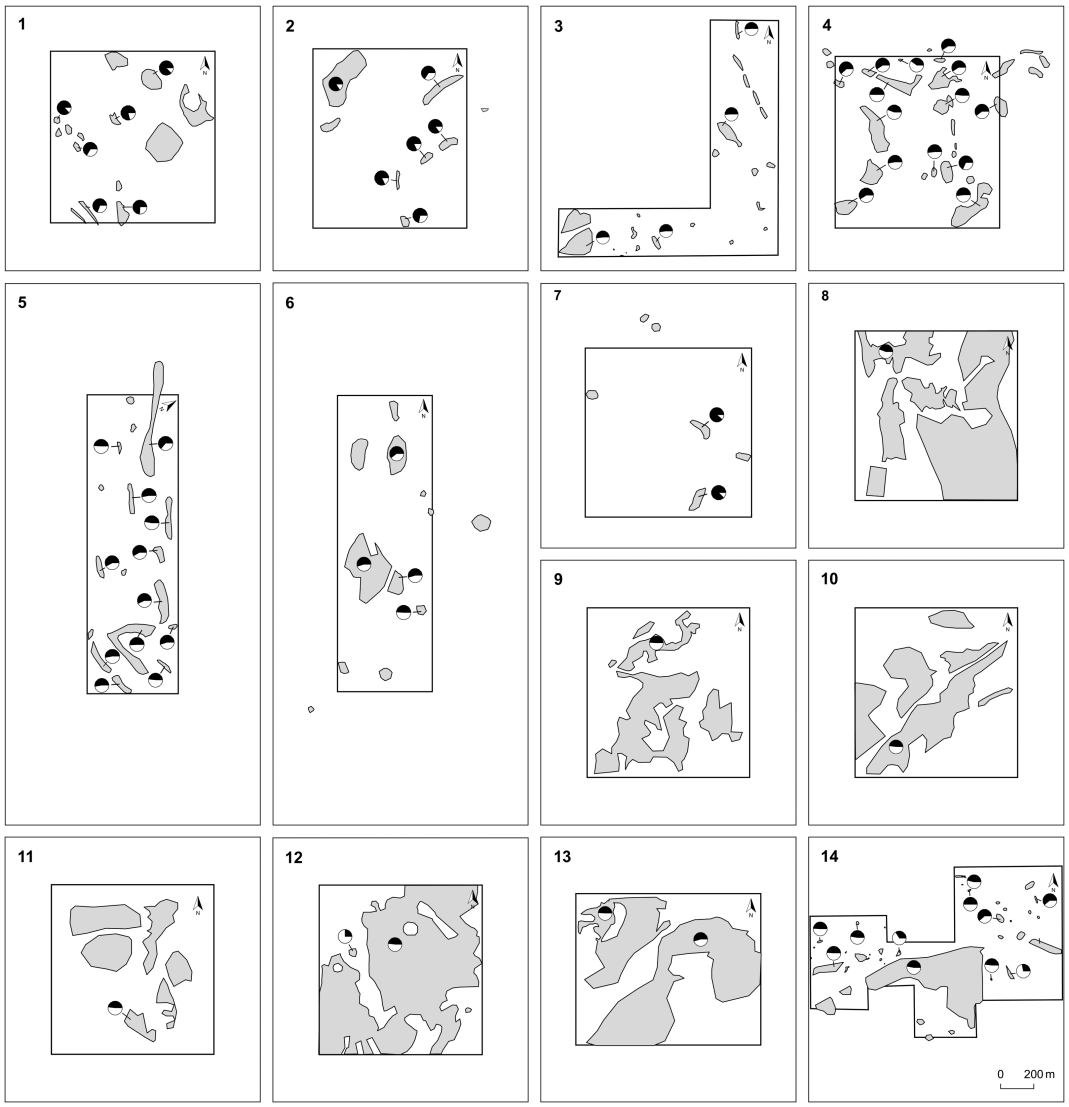

Figure 3: spatial distribution of population patches of *Narcissus assoanus* in 14 selected zones in the study area (locations of zones shown in Fig. 2). Polygons (population patches) outside the 1 km^2 study zone were only used to calculate the isolation index for population patches within study zone.

The spatial configuration of patches in each of the 1 km^2 zones was performed by integrating their GPS coordinates (accuracy < 10 m) into ArcView 10.0. With the aid of topographical maps and orthorectified digital photographs for interpretation, each patch was delimited and its surface area quantified. Once the population patches were delimited, they were recorded in a PostGIS database, a geospatial extension of PostgreSQL and the minimum distances to the neighbouring patches were calculated by means of a Spatial SQL script, using the ST_Distance function (http://www.postgis.org/docs/ST_Distance.html). Finally, we selected the three shortest distances for each population patch to other patches using the Delaunay triangulation method.

In the 14 zones, morph ratios were sampled in 1–14 patches with a mean of 105 plants (range 33–336) per patch. As above, the different zones were visited in 2–3 different years (during the period 2001–2006) to check that all patches were included in our analysis of a given zone and also to verify that morph ratios do not vary with differences in flowering abundance in different years. Based on their morph ratio, patches were classified as biased or isoplethic, depending on whether or not the morph ratio showed a significant departure from a 1:1 ratio. For some patches, it was impossible to precisely define their spatial limits and be sure that they are not continuous with other patches. Hence, in a small number of the 14 zones, a single morph ratio in a single spatially extensive patch was obtained. In spatially extensive patches, we sampled morph ratios in an area equivalent to less extensive patches.

For each of the 1 km^2 zones, we recorded the following variables: patch surface area and mean distance to the closest neighbouring patch (either within or outside the perimeter of the zone), the total number of patches, the

total occupied surface area and the number of biased and isoplethic morph ratios. We used patch surface area as an estimator of population size, based on a significant correlation found between patch area and the estimated number of individuals in the patch (Spearman correlation, $\rho = 0.85$, $P < 0.01$, $n = 9$). In addition, three categories were established: (i) 'isoplethic zones' in which all patches have isoplethic morph ratios; (ii) 'biased zones' in which all patches have biased morph ratios and (iii) 'mixed zones' containing both isoplethic and biased patches. In zone 12, two patches were recorded for morph ratio, one isoplethic patch that covers most of the zone and one very small S-morph biased patch. Because of the prevalence of isoplethy for the numerous morph ratio counts made across the large patch (J. D. Thompson, unpublished data), we considered this an isoplethic zone.

A patch isolation index (S) was calculated with modifications following Hanski and Thomas (1994) as:

$$S_i = \sum e^{-d_{ij}} A_j$$

The sum is calculated over all population patches $j \neq i$ where the species is present, d_{ij} is the distance between patches i and j and A_j is the surface area of the patch j. This index allows for the combined analyses of distance and area effects: isolation is a function of the matrix of pairwise distances between population patches and the surface area of neighbouring patches, but not the surface area of the given patch itself. This is an important point because it means that independent tests of patch surface area (estimator of population patch size) and distance can be made in addition to a test of the isolation index that is not directly based on the two variables. The smaller the S_i values the greater the isolation of a given population patch. Among the different possibilities of assessing landscape connectivity (Wagner and Fortin 2005), we chose this index because it has been used to test the effects of landscape fragmentation in different organisms (Hokit *et al.* 1999; Winfree *et al.* 2005), including Lepidoptera (Brückmann *et al.* 2010; Krauss *et al.* 2003b; Steffan-Dewenter and Tscharntke 2000), which are the main pollinators of *N. assoanus* (Baker *et al.* 2000a).

Within-population scale

To quantify any very localized spatial heterogeneity in morph ratios, we sampled morph ratios in five randomly placed quadrats (each of 2 × 2 m) in each of 19 population patches. In each quadrat, we recorded the floral morph of all flowering stems (mean number of flowers per quadrat = 107, range 14–510). These data were also used to provide an estimate of flower density within population patches of different spatial extent and to examine pollinator behaviour (see below).

Pollinator observations and maternal fecundity

We selected eight patches for pollinator observations, two in each of four different zones that cover the range of patch sizes, morph ratios and geographic location in the study area (zones 1, 3, 12 and 14). In each patch, pollinator activity was recorded by observing pollinator visits to flowers in 5 × 5 m plots with abundant flowers during individual 30-min surveys. All surveys (or records) were done between 10 and 16 h on warm sunny days. A total of 41 h of observations were performed in 2012. We recorded pollinator identity and three components of their behaviour when visiting flowers: the number of visited flowers per observed insect, the distance travelled by each insect between two successive flowers and the total foraging area of an insect during the 30-min period. These data provide an indication of possible influence of differences in pollinator behaviour that may eventually contribute to observed spatial patterns of morph ratio variation.

For analyses of pollinator observations, each patch was classified as either a small (<1000 m^2), medium-sized (1000–10 000 m^2) or spatially extensive (>10 000 m^2) population. We used these classes because pollinator observations take a lot of time and could not be done in a large number of populations of different sizes. The eight patches are part of the 19 patches in which we quantified morph ratios in five 2 × 2 m quadrats. We used the floral counts in these 19 patches to estimate flower density in the three types of population patches.

To quantify maternal reproductive success in the two morphs (in 2012), fruit and seed set were recorded in 10 patches occurring in seven different zones (numbers 1, 2, 3, 6, 12, 13, 14) that cover the range of patch sizes, morph ratios and geographic location and include patches used for pollinator observations.

Statistical analysis

We tested for differences between observed morph ratio and an expected 1:1 morph ratio using likelihood ratio tests (G-tests) following Sokal and Rohlf (2012). At the regional scale, Spearman correlations were performed between morph ratio (proportion of the L-morph in the morph ratio count) and both latitude and longitude. To check for a non-random distribution of populations in relation to morph ratio, a Moran's test for spatial autocorrelation was performed (Cliff and Ord 1981), using the 'spdep' package in R. These spatial autocorrelations were performed for the whole data set (n = 144 populations) and with the subset of populations that occur in lowland garrigues (n = 114 populations).

At the landscape scale, multiple logistic regression analyses were carried out to identify the relative contribution of the potential explanatory variables such as elevation zone (lowland garrigues vs. upland plateaux), distance to the nearest patch, patch size (its surface area) and the isolation index on the deviation of morph ratios from 1:1. Differences in total occupied surface area in the 1 km^2 study zones, mean patch surface area, mean distance to the nearest population patch and mean isolation index between isoplethic and biased morph ratio landscapes were tested with one-way ANOVA and *post hoc* Tukey Honestly-significant-difference (HSD) comparisons when a significant ANOVA effect was detected.

We also tested for different patterns among and within the lowland garrigues area and the upland plateaux.

The relationships between patch surface area and number of visited flowers and distance travelled by pollinators during two consecutive flower visits were tested with Spearman rank correlations for each of the main types of pollinators. To test for differences in population densities in the different classes of patch size that could influence pollinator behaviour we performed a linear mixed model analysis with patch size as a categorical factor, and population as a random factor, using the 'lmer" function in the 'lme4' package in R. We also performed a Spearman correlation between the subset of populations included in the landscape analysis, whose surface area was known, and mean flower density in a patch.

To test for differences in fruit and seed set, we performed a generalized mixed model with a binomial distribution using the 'glmer' function in the 'lme4' package in R. In this analysis floral morph, morph ratio (isoplethic or biased), and their interaction were treated as fixed factors. Population patch was included in the analyses as a random factor.

For linear models, assumptions were checked by performing a Shapiro–Wilk tests for normality of the residuals, a Harrison–McCabe test for the independence of residuals and a Durbin–Watson test for the homoscedasticity of the residuals. When necessary, log or arcsine transformations were made to fit linear model assumptions. All analyses were performed in R 3.2.0 (R Core Team 2013).

RESULTS

Regional scale

Morph ratios were either isoplethic (n = 86 populations), significantly L-morph biased (n = 54 populations always in lowland garrigues) or significantly S-morph biased (n = 4). In the populations where morph ratio was assessed in different years, we did not observe any significant variation in morph ratios between the two recordings: all populations with a significantly biased morph ratio remained biased and all populations initially sampled with a morph ratio not significantly different from 1/1 maintained an isoplethic morph ratio. We used the most recent morph ratio estimates in all analyses and figures.

The spatial autocorrelation analysis showed that the spatial pattern of populations in relation to morph ratio (measured as % L-morph) is statistically significant (Moran's I = 0.434, P < 0.0001). Thus, populations of L-biased morph ratios tended to be spatially aggregated. In fact, there were significant correlations between morph ratio and latitude (ρ = −0.34, n = 144, P < 0.0001), longitude (ρ = 0.56, n = 144, P < 0.0001) and elevation (ρ = −0.56, n = 144, P < 0.0001). As found in previous work at this spatial scale (Baker *et al.* 2000b), populations located towards the north and west of the study area occur on upland limestone plateaux (> 500 m elevation) and tend to have isoplethic morph ratios, whereas populations to the south and east of the study area occur in lowland garrigues (< 500 m elevation) and have either isoplethic or L-morph biased morph ratios (Fig. 2). In lowland garrigues at < 500 elevation 50% of the 114 sampled populations had L-morph biased ratios, while on upland plateaux at > 500m only 10% of the 30 morph ratios were biased. In an analysis limited to populations in lowland garrigues, the spatial autocorrelation of the populations in relation to morph ratio remained significant (Moran's I = 0.433, P < 0.0001), as did correlations with latitude (ρ = 0.26, n = 114, P < 0.005), longitude (ρ = 0.43, n = 114, P < 0.0001) and elevation (ρ = 0.41, n = 114, P < 0.0001). Hence, in the subset of lowland garrigues populations, morph ratios tend to be more often L-morph biased towards the south-east, i.e. closer to the major sites of human population development around Montpellier (Fig. 2).

Landscape scale

Population patches of *N. assoanus* occupied a surface area in the studied 1 km^2 zones which ranged from less than 1.8 ha in zone 7 to more than 50 ha in zones 8 and 12 (Table 1). The mean distance between patches ranged from 38 m in zone 13 to 462 m in zone 7 (Table 1; see also online Fig. S1). Seven zones contained patches with isoplethic morph ratios, three had only L-morph biased patches, and four had both types of patches ('mixed zones'; Fig. 3). Zones with isoplethic morph ratios were mostly located on the upland plateaux (zones 8–13, Fig. 3), except for zone 3 that occurs in lowland garrigues. On the upland plateaux, occupied surface area by *N. assoanus* in 1 km^2 was significantly higher than in lowland garrigues (F = 20.09, df = 1, P < 0.001; Fig. 3). As a result, the total spatial area occupied by *N. assoanus* in a given landscape is significantly higher where isoplethic morph ratios occur. Most of the biased morph ratios concern an L-morph bias (n = 21), except for two small patches in zone 14 and one small patch in each of zones 4 and 12 that had S-morph biased morph ratios. There were significant differences among L-morph biased, mixed and isoplethic zones for the total occupied surface area (F = 5.39, df = 2, P < 0.05), the mean distance to the nearest patch (F = 6.03, df = 2, P < 0.05) and the mean isolation index (F = 5.11, df = 2, P < 0.05). Tukey HSD *post hoc* comparisons revealed that, when compared with L-morph biased zones, in isoplethic zones populations showed significantly (P < 0.05) lower distances to the nearest population (mean ± SE: 324±78 and 108±29 m, respectively), significantly higher occupied surface area (5.9±2.2 and 35.8±7.5 ha, respectively) and significantly higher mean values of the isolation index (16.9±4.6 and 358.2±52.2, respectively). Mixed morph ratio landscapes showed values that were intermediate between and not significantly different from isoplethic or L-morph biased zones (Tukey HSD, P > 0.05).

Multiple logistic regression analysis revealed that while population patch surface area is not a good predictor of population patch morph ratio, elevation zone, isolation index and to a lesser extent distance to the nearest patch make a significant contribution to the occurrence of L-morph frequency in population patches (Table 2). When analysing each of the two elevation zones separately, distance to the nearest patch and isolation also contributed significantly

Table 1: characteristics of the fourteen 1 km^2 zones in which morph ratio was analysed at the 'landscape scale'

Code	Name	Habitat	Area occupied by patches (m^2)	Mean distance to closest neighbour (m)	Number of patches	Number of morph ratios	Number of biased and isoplethic patches	Landscape type
1	St. Clement	Garrigues	90 967	192	14	6	6:0	Biased
2	Restinclièrs	Garrigues	71 242	320	7	6	6:0	Biased
3	Rouet	Garrigues	63 477	192	14	4	0:4	Isoplethic
4	Aerodrome	Garrigues	117 016	181	27	14	5:9	Mixed
5	Cabanne	Garrigues	100 523	162	17	12	1:11	Mixed
6	Ceyrac	Garrigues	120 787	331	12	4	1:3	Mixed
7	Corconne	Garrigues	17 396	462	6	2	2:0	Biased
8	La Trivale	Plateaux	531 004	145	5	1	0:1	Isoplethic
9	Lavagne	Plateaux	276 093	102	5	1	0:1	Isoplethic
10	Ferrusac	Plateaux	326 668	90	5	2	0:2	Isoplethic
11	St. Pierre de la Fage	Plateaux	205 111	107	6	1	0:1	Isoplethic
12	Rogues	Plateaux	631 742	46	2	2	1:1	Isoplethic
13	Rigalderie	Plateaux	472 594	38	2	1	0:1	Isoplethic
14	Perrarines	Plateaux	195 138	103	33	12	4:8	Mixed

Table 2: multiple logistic regression analysis of morph ratio variation (number of plants of each morph in each patch) in relation to potential predictor variables

Data	Source	Estimate	SE	t	P
Total data set	Intercept	0.221	0.0493	4.482	<0.0001
	Elevation zone	−0.332	0.0639	−5.203	<0.001
	Distance	0.002	4.54 e^{-4}	4.66	<0.0001
	Area	2.98 e^{-7}	2.62 e^{-7}	1.138	0.255
	Isolation	−1.07 e^{-6}	2.65 e^{-7}	−4.045	<0.001
Garrigues					
	Intercept	0.383	0.081	4.748	<0.001
	Distance	0.003	5.08 e^{-4}	5.394	<0.001
	Area	−3.45 e^{-7}	2.82 e^{-6}	−0.122	0.903
	Isolation	−7.99 e^{-6}	1.87 e^{-6}	−4.278	<0.001
Upland plateaux					
	Intercept	−0.104	0.096	1.084	0.278
	Distance	0.002	0.001	−1.553	0.120·
	Area	1.10 e^{-7}	2.70 e^{-7}	0.407	0.684
	Isolation	−1.131 e^{-6}	2.739 e^{-7}	−4.129	<0.001

Distance: distance to nearest population patch. Area: patch surface area. Isolation: population isolation index (S, see Material and Methods for further details). Elevation zone: lowland garrigues versus highland plateaux. Analyses were performed in the total set of studied populations as well as in the subset corresponding to the two elevation zones: lowland garrigues and highland plateaux.

to explain morph ratio variation in garrigues. However, in highland plateaux, the only significant explanatory variable was the isolation index.

Within-population scale

Twelve of the 19 studied populations showed a significant G *heterogeneity* value in the *G*-test (see Table 3) due to the fact that some quadrats were isoplethic and some quadrats had a biased morph ratio or because all quadrats were L-morph biased but to a different degree. Six of the nine globally isoplethic populations had quadrats with both isoplethic and biased morph ratios and only three isoplethic populations had quadrats with consistently isoplethic morph ratios.

Flower density

There was significant variation in flower density among populations (F = 107.5, df = 19, P < 0.001) with significant variation in flower densities in the three classes (F = 5.37, df = 2, P < 0.05). Means tests revealed no significant differences (Tukey test, P > 0.05) between intermediate and large populations (mean ± SE: 34.4 ± 5.1 and 32.2 ± 3.8 flowers/m^2, respectively) and a significant difference (Tukey test, P < 0.05) between each of these classes and small populations (11.9 ± 1.3 flowers/m^2). Flower density was more variable in intermediate and large populations (range 7–72 and 15–60 flowers/m^2, respectively) than in small populations (range 6–21 flowers/m^2). In the subset of populations whose surface area was known, there was a significant correlation between mean flower density and patch surface area (ρ = 0.82, n = 9, P < 0.01).

Pollinator visitation

A total of 170 insects belonging to Lepidoptera, Hymenoptera and Diptera were observed visiting flowers of *N. assoanus*. The most abundant flower visitor was *Goenopteryx cleopatra* (Lepidoptera, Pieridae), which accounted for 58% of observed insect visits (hereafter 'records'; n = 98; Table 4), followed by *Anthophora plumipes* (Hymenoptera, Apidae), that accounted for 14% of records (n = 24; Table 4) and *Macroglossum stellatarum* (Lepidoptera, Sphingidae) (9 records, 5% of insect visitors Table 4). However, the extremely high number of visited

Table 3: morph ratios (proportion of the L-morph) in five 2×2 m quadrats and overall population morph ratio for 19 populations of *N. assoanus* (see Fig. 2) in the different zones (number in parentheses) and parts of the study region (G: garrigues or P: upland plateaux)

		Replicates						
Population	Location	1	2	3	4	5	Population	Heterogeneity
Aerodrome (4)	G	0.63*	0.52	0.40*	0.64*	0.63*	0.55	**
Aguzan	G	0.45	0.45	0.56	0.43	0.47	0.47	ns
Arboras	G	0.55	0.50	0.69**	0.69***	0.64*	0.63***	*
Argeliers	G	0.42	0.57	0.42	0.81***	0.73***	0.60	***
Ceyrac (7)	G	0.37***	0.46	0.52	0.52	0.55	0.47	*
Col de l'Hortus	G	0.92***	0.78***	0.76***	0.82***	0.76***	0.80***	ns
Colline Pompignan	G	0.69***	0.74***	0.46	0.52	0.63***	0.63***	***
Gardiol	G	0.58	0.61	0.39	0.51	0.50	0.53	ns
Pompignan	G	0.47	0.51	0.79***	0.45	0.52	0.54	***
Pont de Bonniole	G	0.58***	0.69***	0.85***	0.75***	0.69***	0.68***	***
Restinclières (2)	G	0.97***	0.70*	0.86***	0.87***	0.90***	0.85***	*
St Bauzille de Montmel	G	0.86***	0.82**	0.86**	0.83**	0.71**	0.80***	ns
St Clément (1)	G	0.65	0.59	0.80***	0.47	0.60	0.62***	*
Le Grand Pâtus	G	0.83***	0.76***	0.82**	0.82**	0.90***	0.82***	ns
La Trivalle (8)	P	0.58	0.50	0.47	0.34*	0.54	0.49	ns
Ferrusac (10)	P	0.72***	0.36	0.52	0.40	0.47	0.50	***
Perrarines (14)	P	0.44	0.51	0.60	0.52	0.47	0.49	ns
Rigalderie (13)	P	0.45	0.41**	0.55	0.37**	0.40***	0.44***	**
Rogues (12)	P	0.29***	0.53	0.41*	0.45*	0.41*	0.42***	***

G tests were used to assess a significant deviation from a 1:1 ratio within and among (heterogeneity) replicates.
*$P < 0.05$; **$P < 0.01$; **$P < 0.001$.

flowers per record for the latter species (up to 520 flowers in one single record during a 30-min period and a mean ± SE of 151±48 flowers), that was statistically higher than any other visitor ($F = 18.65$, $df = 5$, $P < 0.001$), indicates that this hawk-moth could be a main pollinator of *N. assoanus* in the study region. The other main pollinators (*G. cleopatra* and *A. plumipes*) visited a mean ± SE of 32±4 and 27±6 flowers, respectively. If we group all studied populations, the mean number of visited flowers per hour (mean number of visits*mean number of visited flowers), was 75 for *G. cleopatra*, 32 for *M. stellatarum* and 14 *A. plumipes*. Other observed insect visitors belonged to the genus *Bombylius* (Diptera), *Xylocopa*, *Lasioglossum* and *Apis* (Himenoptera) and *Vanessa* and *Zygaena* (Lepidoptera; Table 4), all of them visiting on average < 8 flowers per visit for the Hymenoptera and < 4 flowers per visit for the Diptera and Lepidoptera.

No variation in pollinator composition was detected among studied patches. The three main pollinators were present in most patches, except for *M. stellatarum,* that was not observed in three small populations (one isoplethic and two L-morph biased) and one large isoplethic population (Table 4). Pollinator abundance oscillated between zero and 2–3 individuals per hour, except for *G. cleopatra* in spatially extensive populations where we observed a mean value of up to 5.6 (zone 12) and 6.3 (zone 14) individuals per hour (Table 4).

G. cleopatra butterflies visited fewer flowers per plot in smaller patches than in larger patches ($\rho = 0.34$, $n = 56$, $P < 0.01$, Table 4) and they travelled greater distances between consecutive flowers in larger populations ($\rho = 0.31$, $n = 1848$, $P < 0.001$). The other main pollinators (*A. plumipes* and *M. stellatarum*) travelled similar distances between consecutive flowers independently of patch size ($\rho = 0.03$, $n = 678$, $p = 0.441$ and $\rho = 0.001$, $n = 1504$, $P = 0.970$, respectively). *A. plumipes* (but not *M. stellatarum*) showed a significant correlation between number of visited flowers and patch size ($\rho = 0.54$, $n = 26$, $P < 0.05$).

Maternal reproductive success

In general, fruit set was similar ($\chi^2 = 1.30$, $df = 1$, $P > 0.2$) in L- and S-morph plants (0.79±0.02 and 0.82±0.02, respectively). Fruit set was lower in isopletic than in biased populations although this effect was only marginally significant ($\chi^2 = 2.98$ $df = 1$, $P = 0.084$) and no doubt due to a generally lower fruit set un upland populations because no variation was detected in the subset of garrigues ($\chi^2 = 0.09$, $df = 1$, $P = 0.767$). There was no overall significant interaction between flower morph and morph ratio ($\chi^2 = 2.67$, $df = 1$, $P = 0.102$). In isoplethic populations, there were no significant differences in fruit set between morphs ($\chi^2 = 0.08$, $df = 1$, $P > 0.5$), whereas in L-morph biased populations, there was a significantly higher fruit set ($\chi^2 = 3.87$, $df = 1$, $P < 0.05$) of the

Table 4: number of overall visits of each pollinator (a) and number of visits per hour (b) in isoplethic (I) and L-morph biased ratio (B) populations of *Narcissus assoanus* that are one of three sizes: small (1), intermediate (2) or large (3)

Population	Stcle 10	Stcle 02	Rouet 04	Stcle 01	Rouet 05	Rouet 17	Rogues 01	Perrarines 01	Total
Zone	1	1	3	1	3	3	12	14	
Population size	1	1	1	2	2	2	3	3	
Morph ratio	B	B	I	B	I	I	I	I	
Hours of survey	2	4.5	4.5	8	6.5	5	4.5	6	41
(a) Pollinator visits									
Goenopteryx cleopatra	1	15	4	10	2	3	25	38	98
A. plumipes	1	1		16		6		1	24
Macroglossum stellatarum				3	2	4		1	9
Xylocopa sp.					2	1			3
Bombyiliidae spp.	1			1	4	7			13
Other bees		1		4	2	1		2	10
Other butterflies	2	1		1	7				11
Total visits	5	18	4	35	19	22	25	42	170
(b) Visits per hour									
Goenopteryx cleopatra	0.5	3.3	0.9	1.3	0.3	0.6	5.6	6.3	
Anthophora plumipes	0.5	0.2	0.0	2.0	0.0	1.2	0.0	0.2	
M. stellatarum	0.0	0.0	0.0	0.4	0.3	0.8	0.0	0.2	
Total visits	2.5	4.0	0.9	4.4	2.9	4.4	5.6	7.0	

S-morph (0.96 ± 0.02 compared to 0.88 ± 0.03). There were no significant differences in seed set between morphs ($\chi^2 = 0.02$, $df = 1$, $P > 0.5$), between isoplethic and biased morph ratio populations ($\chi^2 = 0.31$, $df = 1$, $P > 0.5$) and their interaction ($\chi^2 = 0.26$, $df = 1$, $P > 0.5$).

DISCUSSION

This study provides a novel analysis of variation in a genetic polymorphism in relation to the spatial configuration and size of populations at nested spatial scales. The results illustrate how population fragmentation—in terms of a combination of increasing distance and small population size—may affect morph-ratio variation. This study thus provides an illustration of how landscape structure may affect metapopulation dynamics in natural plant populations.

Habitat fragmentation and morph ratio variation at different spatial scales

On a regional scale, our results confirm and complement previous work done at this scale (Baker *et al.* 2000b); the majority of upland plateaux populations have isoplethic morph ratios and populations with significant L-morph biased ratios tend to occur in lowland garrigues, particularly towards the south and east, closer to areas of urban development.

The landscape study of 14 zones in these two areas showed that spatially extensive populations have mostly isoplethic morph ratios whereas local populations in discrete patches of the landscape have either biased or isoplethic morph ratios. Previous studies by Arroyo and Dafni (1995) on *N. tazzeta*, Baker *et al.* (2000b) on *N. assoanus* and Arroyo *et al.* (2002) on *N. papyrac*eus, found a positive relation between population size and the frequency of the S-morph. However, in our landscape-scale study, we found that morph ratio variation is more closely related to the spatial isolation of populations in the landscape than to an effect of population patch size. In the more fragmented landscapes of lowland garrigues, the effect of distance to the nearest path also contributed to explain morph ratio variation. The total spatial area occupied by *N. assoanus* in a given landscape is also significantly higher where isoplethic morph ratios occur.

On a third-spatial scale, within individual populations, we found that small patches can have biased morph ratios, even when they occur in spatially extensive populations with an overall isoplethic morph ratio. Hence, if populations become fragmented it is possible that by chance they will have biased ratios even if the original more spatially extensive population had an isoplethic morph ratio. Stehlik *et al.* (2006) reported spatial clustering of morphs in *N. assoanus* at distances less than 5 m (as in our study). Although vegetative propagation (by the division of the underground bulbs in our study species) can contribute to such patterns (e.g. Stehlik and Holderegger 2000), there is no reason why L-morph bulbs should divide more than those of the S-morph.

Plant species with a sexual polymorphism frequently show morph ratio variation across their distribution. Such variation has often been reported when stochasticity associated with colonization by a small number of founder individuals causes the loss or decline in abundance of morphs (Barrett *et al.* 1989; Couvet *et al.* 1986, 1998). Our finding that the evolution of biased frequencies occurs in fragmented populations suggests that stochasticity associated with founder

events during population colonization or fragmentation of existing populations may play important roles in the evolutionary dynamics of morph ratio variation in the study species. In stigma-height dimorphic *N. papyraceus*, dimorphic populations are concentrated in the Strait of Gibraltar, while L-monomorphic populations occur on the northern edge of the range (Arroyo *et al.* 2002), where pollinators that assure the pollination of the S-morph are scarcer (Pérez-Barrales and Arroyo 2010). Populations of *N. tazetta* in Israel in lowland marshes are dominated by the S-morph and in upland areas by the L-morph, a pattern interpreted in the context of pollinator-mediated selection (Arroyo and Dafni 1995). In our study-system, long-tongued pollinators are consistently present, regardless of the population morph ratio (also: J.D. Thompson unpublished observations). We thus have no evidence that the S-morph declines in frequency due to absence of a pollinator that assures its reproduction. Indeed fruit set is not lower in the S-morph (see also Baker *et al.* 2000a, 2000c).

The joint finding that biased morph ratios occur in more isolated populations and in small patches within populations with an overall isoplethic morph ratio suggest that fragmentation and limited seed dispersal may play a major role in the occurrence of such morph ratios. Biased morph ratios may be facilitated by two other factors. First, *N. assoanus* does not have a heteromorphic incompatibility system (Baker *et al.* 2000c; Cesaro *et al.* 2004), hence, while complete dissassortative mating (among morph pollination) will maintain the two morphs at equal frequencies, assortative pollination (i.e. selfing and crosses between plants of the same morph) can lead to rapid evolution of morph ratios (Barrett *et al.* 1996; Baker *et al.* 2000c). Such assortative pollination is likely to be higher in the L-morph because the anthers are closer to the stigma than in the S-morph (Baker *et al.* 2000b; Thompson *et al.* 2012). In addition the latter shows much lower rates of self-pollen deposition than the L-morph (Cesaro *et al.* 2004). This difference may favour pollen transport among plants of the L-morph (Cesaro and Thompson 2004; Thompson *et al.* 2003). Second, the style-morph inheritance of *N. assoanus* is assumed to be determined by single locus, as in stigma-height dimorphic *N. tazetta* (Dulberger 1964), with the L-morph determined by recessive alleles (ss) and the S morph by a dominant allele (Ss). This genetic control by a dominant allele may further contribute to reduced frequency of the S-morph (Baker *et al.* 2000c); when a stochastic loss of the 'S-allele' occurs this would facilitate evolution of morph ratios and a more frequent bias in morph ratios towards higher frequencies of the L-morph (whose genetic control does not allow such stochastic loss).

Foraging by insect pollinators can also depend on the patch size and spatial clustering of flowering plants, as found in studies of bumblebee pollinator visits in small fragmented populations which are less frequent but longer than in larger populations (Goverde *et al.* 2002; Sowig 1989). This pattern can be explained as a compensation of energy lost during flights among patches (Goverde *et al.* 2002) or as a response based on the capacity to respond to local reward levels by making shorter flights in relation to the spatial organization of flowers (Cartar and Real 1997; Cresswell 1990, 2000; Sowig 1989). In our study we observed differential patterns of foraging behaviour of the main floral visitor of *N. assoanus* (the butterfly *G. cleopatra*) in different population contexts. In spatially extensive populations, this species is observed to fly several metres during consecutive visits even across dense patches of flowers. In contrast, in smaller and more isolated populations, this species tends to consecutively visit nearby flowers and to repeatedly move back into the population from the edge. In small populations, floral density is on average less than that in intermediate and large populations in our study populations. Hence, despite a high local density of flowers, in large populations pollinators tend to fly larger distances among consecutive flowers (see also Rasmussen and Brodsgaard 1992). These observations were made in a single year in a small number of populations (due to unfavourable meteorological conditions), hence we are very cautious in interpreting their significance. They do however follow the idea that pollen dispersal will tend to be more localized in small local populations, creating the conditions for assortative mating and the development of biased morph ratios.

Towards isoplethy: frequency dependent selection

Although L-monomorphic populations exist in other *Narcissus* species (Arroyo *et al.* 2002; Baker *et al.* 2000b; Thompson 2005), no monomorphic populations have been observed for *N. assoanus* in our study region, elsewhere in southern France (J. Thompson unpublished observations) or in Spain (Thompson *et al.* 2012). Thus, in *N. assoanus*, the mechanisms leading to L-morph biased populations do not lead to complete loss of the S-morph from natural populations, despite their rarity in some populations and despite computer models that indicate loss of the S-morph as the proportion of assortative mating increases (Baker *et al.* 2000c).

In experimental arrays with variable morph ratios in a natural population with an isoplethic morph ratio (Thompson *et al.* 2003), we have reported that S-morph plants showed a significantly higher fruit set than L-morph plants in L-morph biased populations, while in isoplethic arrays, both morphs exhibited similar fruit set. This result has been observed in the present study of natural populations, but not in other studies of natural populations (Baker *et al.* 2000a, 2000c). Using neighbourhood models, Stehlik *et al.* (2006) also found evidence of frequency-dependent variation of female fertility in the S-morph in natural populations of *N. assoanus*. Thus, in some years and population contexts the S-morph may be subject to negative frequency dependent selection when rare (Fisher 1930).

Negative frequency-dependent selection may be facilitated in *N. assoanus* by efficient between-morph pollination transfer from L-morph donors to S-morph receivers, which set more seed in the presence of L-morph pollen donors than in the presence of S-morph pollen donors (Cesaro and Thompson 2004;

Thompson *et al.* 2012). The floral morphs in *Narcissus* are thus not functionally equivalent as mating partners, most probably due to enhanced reciprocity between the height of the S-morph stigma and the lower anthers of the L-morph (Thompson *et al.* 2012). Stochastic deviation of morph ratios towards L-morph biased ratios may thus be brought back towards isoplethy by frequency-dependent selection in association with subtle differences among morphs in their floral morphology. This could explain the absence of monomorphic L-morph populations and the maintenance of the polymorphism in all populations of the study species that we have encountered in 20 years of observations in Mediterranean France and Spain.

Concluding remarks

In plants there has been considerable debate around the idea that spatial population structure causes a dissociation of processes operating at the different spatial scales that characterize metapopulation dynamics (Ehrlén and Eriksson 2003; Freckleton and Watkinson 2002; Pannell and Obbard 2003). Our study illustrates that the discrete local populations in lowland garrigues show several criteria for their definition as a regional metapopulation (Freckleton and Watkinson 2002): local populations are not too isolated to show recolonization events (our landscape scale study), occur in discrete patches among which gene flow is limited (distance among populations contributes to patterns of morph ratio variation) with high levels of pollination among neighbouring plants (pollinator observations), and show asynchronous dynamics (morph ratios vary considerably in a landscape setting indicative that populations may differ in age or time since fragmentation/colonization). This interpretation bears a striking similarity to hypotheses for sex ratio variation in gynodioecious *Thymus vulgaris* which co-occurs with *N. assoanus*, very often in the same sites (Couvet *et al.* 1986, 1998).

In contrast, in a landscape context of largely uninterrupted habitat of extensively grazed rocky limestone pastures, *N. assoanus* does not appear to function as a metapopulation (see Murphy and Lovett-Doust 2004). Here, 'spatially extended' (*sensu* Freckleton and Watkinson 2002) and almost continuous populations occur across large areas of suitable habitat where, as the latter authors have argued (page 419), 'regional dynamics … (may be) … essentially a simple extension of local dynamics'. The fact that populations of *N. assoanus* are to a large majority isoplethic in this area indicates that the local dynamics associated with natural selection on morph ratio frequencies are indeed extended across the landscape.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Plant Ecology* online.

FUNDING

French 'Agence Nationale de la Recherche' (contract 05-BDIV-014, ABIME). Spanish 'Ministerio de Ciencia e Innovación' and ERDF projects (CGL2009-08257 and CGL2012-33270), and a postdoctoral contract (EX2009-0751) awarded to RB by the Spanish 'Ministerio de Educación'.

ACKNOWLEDGEMENTS

We thank C. Bernard and A. Letourneau for help with ArcGIS and PostGIS, F. Balao for statistical advice and M. Arista, J. Arroyo and two anonymous reviewers for comments that helped to improve a preliminary version of the manuscript.
Conflict of interest statement. None declared.

REFERENCES

Aguilar R, Ashworth L, Galetto L, *et al.* (2006) Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. *Ecol Lett* **9**: 968–80.

Aizen MA, Ashworth L, Galetto L (2002) Reproductive success in fragmented habitats: do compatibility systems and pollination specialization matter? *J Veg Sci* **13**:885–92.

Arroyo J, Barrett SCH, Hidalgo R, *et al.* (2002) Evolutionary maintenance of stigma-height dimorphism in *Narcissus papyraceus* (Amaryllidaceae). *Am J Bot* **89**:1242–9.

Arroyo J, Dafni A (1995) Variations in habitat, season, flower traits and pollinators in dimorphic *Narcissus tazetta* L. (Amaryllidaceae) in Israel. *New Phytol* **129**:135–45.

Arroyo-Rodríguez V, Pinuead E, Escobar F, *et al.* (2009) Value of small patches in the conservation of plant-species diversity in highly fragmented rainforest. *Conserv Biol* **23**: 729–39.

Ashworth L, Aguilar R, Galetto L, *et al.* (2004) Why do pollination generalist and specialist plant species show similar reproductive susceptibility to habitat fragmentation? *J Ecol* **92**:717–9.

Baker AM, Barrett SCH, Thompson JD (2000a) Variation of pollen limitation in the early flowering Mediterranean geophyte *Narcissus assoanus* (Amaryllidaceae). *Oecologia* **124**:529–35.

Baker AM, Thompson JD, Barrett SC (2000b) Evolution and maintenance of stigma-height dimorphism in *Narcissus*. I. Floral variation and style-morph ratios. *Heredity* **84**:502–13.

Baker AM, Thompson JD, Barrett SCH (2000c) Evolution and maintenance of stigma-height dimorphism in *Narcissus*. II. Fitness comparisons between style morphs. *Heredity* **84**:514–24.

Barrett SCH, Lloyd DG, Arroyo J (1996) Stylar polymorphisms and the evolution of heterostyly in *Narcissus* (Amaryllidaceae). In Lloyd DG, Barrett SCH (eds). *Floral Biology. Studies on Floral Evolution in Animal-Pollinated Plants*. New York: Chapman & Hall, 339–76.

Barrett SCH, Morgan M, Husband BC (1989) The dissolution of a complex genetic polymorphism: the evolution of self-fertilization in tristylous *Eichhornia paniculata* (Pontederiaceae). *Evolution* **43**:1398–1416.

Bernhardt CE, Mitchell RJ, Michaels HJ (2008) Effects of population size and density on pollinator visitation, pollinator behavior, and pollen tube abundance in *Lupinus perennis*. *Int J Plant Sci* **169**:944–53.

Brückmann SV, Krauss J, Steffan-Dewenter I (2010) Butterfly and plant specialists suffer from reduced connectivity in fragmented landscapes. *J Appl Ecol* **47**:799–809.

Cartar RV, Real LA (1997) Habitat structure and animal movement: the behaviour of bumble bees in uniform and random spatial resource distributions. *Oecologia* **112**:430–4.

Cesaro AC, Barrett SCH, Maurice S, *et al.* (2004) An experimental evaluation of self-interference in *Narcissus assoanus*: functional and evolutionary implications. *J Evol Biol* **17**:1367–76.

Cesaro AC, Thompson JD (2004) Darwin's cross-promotion hypothesis and the evolution of stylar polymorphism. *Ecol Lett* **7**:1209–15.

Chetkiewicz CLB, Clair CCS, Boyce MS (2006) Corridors for conservation: integrating pattern and process. *Ann Rev Ecol Evol Syst* **37**:317–42.

Cliff AD, Ord JK (1981) *Spatial Processes, Models and Applications*. London: Pion.

Couvet D, Bonnemaison F, Gouyon PH (1986) The maintenance of females among hermaphrodites: the importance of nuclear-cytoplasmic interactions. *Heredity* **57**:325–30.

Couvet D, Ronce O, Gliddon C (1998) The maintenance of nucleocytoplasmic polymorphism in a metapopulation: the case of gynodioecy. *Am Nat* **152**: 59–70.

Cozzi G, Muller CB, Krauss J (2008) How do local habitat management and landscape structure at different spatial scales affect fritillary butterfly distribution on fragmented wetlands? *Landsc Ecol* **23**:269–83.

Cresswell JE (1990) How and why do nectar-foraging bumblebees initiate movements between inflorescences of wild bergamot *Monarda fistulosa* (Lamiaceae)? *Oecologia* **82**:450–60.

Cresswell JE (2000) A comparison of bumblebees' movements in uniform and aggregated distributions of their forage plant. *Ecol Entomol* **25**:19–25.

Damschen EI, Brudvig LA, Haddad NM, *et al.* (2008) The movement ecology and dynamics of plant communities in fragmented landscapes. *Proc Natl Acad Sci USA* **105**:19078–83.

Dauber J, Biesmeijer JC, Gabriel D, *et al.* (2010) Effects of patch size and density on flower visitation and seed set of wild plants: a pan-European approach. *J Ecol* **98**:188–96.

Dulberger R (1964) Flower dimorphism and self-incompatibility in *Narcissus tazetta* L. *Evolution* **18**:361–3.

Eckert CG, Barrett SCH (1992) Stochastic loss of style morphs from populations of tristylous *Lythrum salicaria* and *Decodon verticillatus* (Lythraceae). *Evolution* **46**: 1014–29.

Ehrlén J, Eriksson O (2003) Large-scale spatial dynamics of plants: a response to Freckleton and Watkinson. *J Ecol* **91**:316–20.

Endels P, Jacquemyn H, Brys R, *et al.* (2002) Temporal changes (1986–1999) in populations of primrose (*Primula vulgaris* Huds.) in an agricultural landscape and implications for conservation. *Biol Conserv* **105**:11–25.

Fahrig L (2003) Effects of habitat fragmentation on biodiversity. *Ann Rev Ecol Evol Syst* **34**:487–515.

Fisher RA (1930) *The Genetical Theory of Natural Selection*. New York, NY: Dover Publications.

Freckleton RP, Watkinson AR (2002) Large-scale spatial dynamics of plants: metapopulations, regional assemblages and patchy populations. *J Ecol* **90**:419–34.

Goverde M, Schweizer K, Baur B, *et al.* (2002) Small-scale habitat fragmentation effects on pollinator behaviour: experimental evidence from the bumblebee *Bombus veteranus* on calcareous grasslands. *Biol Conserv* **104**:293–9.

Hanski I, Thomas CD (1994) Metapopulation dynamics and conservation: a spatially explicit model applied to butterflies. *Biol Conserv* **68**:167–80.

Hilty JA, Lidicker WZJ, Merelender AM (2006) *Corridor Ecology. The Science and Practice of Linking Landscapes for Biodiversity Conservation*. Washington, DC: Island Press.

Hodgins KA, Barrett SCH (2008) Asymmetrical mating patterns and the evolution of biased style-morph ratios in a tristylous daffodil. *Genet Res* **90**:3–15.

Hokit DG, Stith BM, Branch LC (1999) Effects of landscape structure in Florida scrub: a population perspective. *Ecol Appl* **9**:124–34.

Husband BC, Barrett SCH (1992a) Genetic drift and the maintenance of the style length polymorphism in tristylous populations of *Eichhornia paniculata* (Pontederiaceae). *Heredity* **69**:440–49.

Husband BC, Barrett SCH (1992b) Pollinator visitation in populations of the tristylous *Eichhornia paniculata* in northeastern Brazil. *Oecologia* **89**:365–71.

Kéry M, Matthies D, Schmid B (2003) Demographic stochasticity in population fragments of the declining distylous perennial *Primula veris* (Primulaceae). *Basic Appl Ecol* **4**:197–206.

Krauss J, Steffan-Dewenter I, Tscharntke T (2003a) How does landscape context contribute to effects of habitat fragmentation on diversity and population density of butterflies? *J Biogeogr* **30**:889–900.

Krauss J, Steffan-Dewenter I, Tscharntke T (2003b) Local species immigration, extinction, and turnover of butterflies in relation to habitat area and habitat isolation. *Oecologia* **137**:591–602.

Lindborg R, Eriksson O (2004) Historical landscape connectivity affects present plant species diversity. *Ecology* **85**:1840–5.

Lindenmayer DB, Fischer J (2007). Tackling the habitat fragmentation panchreston. *Trends Ecol Evol* **22**:127–32.

Matsumura C, Whashitani I (2000) Effects of population size and pollinator limitation on seed set of *Primula sieboldii* populations in a fragmented landscape. *Ecol Res* **15**:307–22.

Murphy HT, Lovett-Doust J (2004) Context and connectivity in plant metapopulations and landscape mosaics: does the matrix matter? *Oikos* **105**:3–14.

Öckinger E, Lindbourg R, Sjödin NE, *et al.* (2011) Landscape matrix modifies richness of plants and insects in grassland fragments. *Ecography* **34**:1–9.

Öckinger E, Smith HG (2006) Landscape composition and habitat area affect butterfly species richness. *Oecologia* **149**:526–34.

Pannell JR, Obbard DJ (2003) Probing the primacy of the patch: what makes a metapopulation. *J Ecol* **91**:485–88.

Pérez-Barrales R, Arroyo J (2010) Pollinator shifts and the loss of style polymorphism in *Narcissus papyraceus* (Amaryllidaceae). *J Evol Biol* **23**:1117–28.

R Core Team (2013) *R: a Language and Environment for Statistical Computing*. *R Foundation for Statistical Computing*. Vienna, Austria. http://www.R-project.org/.

Rasmussen IR, Brodsgaard B (1992) Gene flow inferred from seed dispersal and pollinator behaviour compared to DNA analysis of restriction site variation in a patchy population of *Lotus corniculatus* L. *Oecologia* **89**:277–83.

Sala OE, Chapin FSI, Armesto JJ, *et al.* (2000) Global biodiversity scenarios for the year 2100. *Science* **287**:1770–4.

Santos-Gally R, Perez-Barrales R, Simon VI, *et al.* (2013). The role of short-tongued insects in floral variation across the range of a style-dimorphic plant. *Ann Bot* **111**:317–28.

Sokal RR, Rohlf FJ (2012) *Biometry: the Principles and Practice of Statistics in Biological Research*, 4th edn. New York, NY: WH Freeman and Co.

Sowig P (1989) Effects of flowering plants patch size on species composition of pollinator communities, foraging strategies and resource partitioning in bumblebees (Hymenoptera, Apidae). *Oecologia* **78**:550–8.

Steffan-Dewenter I, Tscharntke T (1999) Effects of habitat isolation on pollinator communities and seed set. *Oecologia* **121**:432–40.

Steffan-Dewenter I, Tscharntke T (2000) Butterfly community structure in fragmented habitats. *Ecol Lett* **3**:449–56.

Steffan-Dewenter I, Tscharntke T (2002) Insect communities and biotic interactions on fragmented calcareous grasslands: a mini review. *Biol Conserv* **104**:275–84.

Stehlik I, Caspersen JP, Barrett SCH (2006) Spatial ecology of mating success in a sexually polymorphic plant. *Proc Royal Soc B* **273**:387–94.

Stehlik I, Holderegger R (2000) Spatial genetic structure and clonal diversity of *Anemone nemorosa* in late successional deciduous woodlands of Central Europe. *J Ecol* **88**:424–35.

Taylor PD, Fahrig L, Henein K, *et al.* (1993) Connectivity is a vital element of landscape structure. *Oikos* **68**:571–3.

Thomas JA, Bourn NAD, Clarke RT, *et al.* (2001) The quality and isolation of habitat patches both determine where butterflies persist in fragmented landscapes. *Proc Royal Soc B Biol Sci* **268**:1791–6.

Thompson JD (2005) *Plant Evolution in the Mediterranean*. Oxford, UK: Oxford University Press.

Thompson JD, Barrett SCH, Baker AM (2003) Frequency-dependent variation in reproductive success in *Narcissus*: implications for the maintenance of stigma-height dimorphism. *Proc Royal Soc B* **270**:949–53.

Thompson JD, Cesaro A, Arroyo J (2012) Morph ratio variation and sex organ reciprocity in style-dimorphic *Narcissus assoanus*. *Int J Plant Sci* **173**:1–10.

Van Rossum F, De Sousa SC, Triest L (2006) Morph-specific differences in reproductive success in the distylous *Primula veris* in a context of habitat fragmentation. *Int J Ecol* **30**:426–33.

Wagenius S (2006) Scale dependence of reproductive failure in fragmented *Echinacea* populations. *Ecology* **87**:931–41.

Wagner HH, Fortin MJ (2005) Spatial analysis of landscapes: concepts and statistics. *Ecology* **86**:1975–87.

Ward M, Johnson SD (2005) Pollen limitation and demographic structure in small fragmented populations of *Brunsvigia radulosa* (Amaryllidaceae). *Oikos* **108**:253–62.

Wilcove DS, McLellan CH, Dobson AP (1986) Habitat fragmentation in the temperate zone. In Soulé ME (ed). *The Science of Scarcity and Diversity*. Sunderland, MA: Sinauer Associates Inc, 237–56.

Winfree R, Dushoff J, Crone EE, *et al.* (2005) Testing simple indices of habitat proximity. *Am Nat* **165**:707–17.

Winter C, Lehmann S, Diekmann M (2008) Determinants of reproductive success: a comparative study of five endangered river corridor plants in fragmented habitats. *Biol Conserv* **141**:1095–104.

Young A, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. *Trends Ecol Evol* **11**:413–18.