RANGE-WIDE VARIATION IN THE ECOLOGICAL NICHE AND FLORAL POLYMORPHISM OF THE WESTERN MEDITERRANEAN GEOPHYTE *NARCISSUS DUBIUS* GOUAN

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Premise of research. Comparative studies of variation in the ecology and genetics of natural plant populations located at the limits and in the center of a species range provide fundamental insights into the historical formation of species distribution patterns.

Methodology. In this study, we quantify variation in the ecological niche and the expression of a floral polymorphism across the range of the Mediterranean geophyte *Narcissus dubius* Gouan. An exhaustive data set of known locations was compiled to distinguish groups of geographically central and peripheral populations in both Spain and France. These occur across a double climatic gradient. First, there is a gradient from a hot and dry Mediterranean climatic regime in eastern Spain to a milder Mediterranean climatic regime in southern France (lower maximum temperature and shorter summer drought). Second, there is a shift to a more continental climate in peripheral populations in inland Spain. We also modeled the current climatic niche and produced historical projections of potential glacial refugia for this species.

Pivotal results. Peripheral populations showed consistent patterns of ecological niche differences with a less rupicolous ecology, more bare soil, and a higher cover of annual species. The ecological niche was more variable among peripheral populations than among central populations. Peripheral populations showed a repeated pattern of loss of stigma-height polymorphism and floral traits indicative of a reproductive strategy based on within-morph mating (probably selfing to assure seed set). Climate models indicate that contemporary peripheral populations (particularly in Spain) occur in areas that were least likely to have served as glacial refugia.

Conclusions. Historical isolation and recolonization may have shaped contemporary patterns of ecological niche and reproductive trait variation among central and peripheral populations.

Keywords: central-peripheral hypothesis, ecological niche, floral polymorphism, Last Glacial Maximum, Mediterranean, refugia.

Online enhancements: appendix figures and tables.

Introduction

Variation in the ecology and genetics of natural plant populations across a species' geographic range is crucial to our understanding of the factors that have shaped current distribution patterns (Gaston 2003) and the processes acting in expanding range limits (Thomas et al. 2001). The comparison of populations in the central part of a species distribution with those in peripheral or (peripherally) isolated parts of a species distribution has been a dominant theme in such work.

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Such studies provide an opportunity to examine variation in the adaptive and nonadaptive processes acting on populations across the range of widespread species.

There has been much interest in how levels of neutral genetic diversity within and among populations may vary from central to peripheral populations. Many studies have indeed shown how levels of genetic variability within populations may decline, despite possible increased differentiation among populations at range limits (Lönn and Prentice 2002; Persson et al. 2004; Hampe and Petit 2005; Eckstein et al. 2006; Eckert et al. 2008; Meeus et al. 2012). In parallel, the comparison with populations in the central part of a species distribution provides insights into how adaptive traits and population dynamics may vary at range limits in relation to differences in

their size and isolation (Brown 1984; Sagarin and Gaines 2002; Herlihy and Eckert 2005; Angert 2006; Villellas et al. 2012). Floral polymorphisms have been particularly interesting here, because variability in the relative frequency of floral types and possible loss of the polymorphism are highly sensitive to variation in both natural selection pressures (Thompson et al. 2003) and nonadaptive stochastic processes (Barrett et al. 1989). Indeed, species with floral polymorphisms often show marked geographic variation in morph ratios across their range, which may be related to variation in adaptive mechanisms linked to changes in pollinator composition (Arroyo and Dafni 1995; Barrett et al. 2004; Hodgins and Barrett 2008; Pérez-Barrales and Arroyo 2010; Santos-Gally et al. 2013) and selection on floral traits that facilitate selfing and associated reproductive assurance in parts of the range where pollination is limited (Eckert and Barrett 1993; Herlihy and Eckert 2005). But this pattern can also be the result of stochastic effects in colonizing populations or past bottlenecks (Couvet et al. 1986; Barrett et al. 1989; R. Berjano, P. Gauthier, V. Pons, and J. D. Thompson, unpublished manuscript).

There has been continued discussion of the idea that geographically peripheral populations may be ecologically marginal (Brown 1984; Abeli et al. 2014), although this has received much criticism (Soule 1973; Kawecki 2008; Martínez-Meyer et al. 2013; Chardon et al. 2014; Lira-Noriega and Manthey 2014; Pironon et al. 2015). In this context, there has been increasing effort to compare the ecological characteristics of central and peripheral populations, either with an approach based on models of the macroniche, primarily based on broad climatic features of the region where populations occur (Martínez-Meyer and Peterson 2006; Diniz-Filho et al. 2009; Lira-Noriega and Manthey 2014), or by empirical investigation of the micro-ecological niche, where plants grow in terms of soil properties (Farris and Schaal 1983; Van Rossum and Prentice 2004; Duffy et al. 2009; Leuschner et al. 2009; Wagner et al. 2011; Wasof et al. 2013), competition, community composition (Carter and Prince 1985; Alexander et al. 2007), herbivory (Bruehlheide and Scheidel 1999; Stanton-Geddes et al. 2012; Castilla et al. 2013), and/or other biotic interactions (Zalewska-Gałosz et al. 2012). To date, no general pattern of converging ecological features toward range margins has been identified (Leuschner et al. 2009).

The contemporary distribution of many western Mediterranean plants provides an ideal situation to conduct range-wide studies of variation in the genetics and ecology of natural plant populations. The Mediterranean region of southern France is particularly interesting here, in that it represents the northern and eastern or western range limits of many Mediterranean plant species (Jahandiez 1937; Quézel and Médail 2003; Noble and Diadema 2011; G. Papuga, unpublished data). The distribution of such species is related to an interactive combination of the geological and climatic histories of the region, which have imposed biogeographic barriers and created climatic conditions for changes in species distribution. They constitute evolutionary processes that are associated with diversification (Thompson 2005). The onset of the Mediterranean climate in the Pliocene, followed by repeated glacial periods in the Pleistocene, has caused repeated range contractions, with persistence in distinct refugia (Carrión et al. 2003; López de Heredia et al. 2007; Médail and Diadema 2009) and subsequent expansion. As a result, the historical center from which

species have spread may not always be the central part of the contemporary geographic range (Carnaval et al. 2009; Lira-Noriega and Manthey 2014). Hence, species history may contribute to the processes underlying contemporary patterns (Hampe and Petit 2005; Pironon et al. 2015).

In this article, we explore variation in a floral polymorphism and the ecological niche of central and peripheral populations of the Mediterranean geophyte, *Narcissus dubius* Gouan. Like many species of its genus, *N. dubius* shows a stigma-height polymorphism, with two morphs that have anthers at roughly the same height and stigmas that are either positioned below the anthers (short-styled S-morph) or level with or above the anthers (long-styled L-morph). Several *Narcissus* species show striking patterns of geographic variation in morph ratios across their range (see references above), and preliminary observations illustrate that this may also be the case for *N. dubius* (Baker et al. 2000a; Thompson 2005). The species occurs across a broad climatic gradient in the Mediterranean region, from southern Catalonia to Provence in southeast France and into the Aragon region of central Spain.

This study has three objectives. First, to assess whether peripheral and central populations of *N. dubius* differ in ecological characteristics, we quantify the broad-scale habitat characteristics (climate parameters and altitude) or macroniche of natural populations and the fine-scaled ecological features of the precise environment in which plants grow (i.e., the microniche). To do so, we compiled a database of known populations and their broad habitat characteristics and performed an empirical investigation of the microniche in two groups of central populations and two groups of geographically peripheral populations (in Aragon to the northwest and in Provence to the northeast). Second, we investigated geographic variation in the floral polymorphism in two ways. We quantified the ratio of morphs in populations throughout the range of the species and assessed the proportion of dimorphic and monomorphic populations in central and peripheral groups. Then, for the L-morph, we quantified variability in stigma-anther separation within and among dimorphic (central) and monomorphic (peripheral) populations to assess any trend toward a floral biology that could facilitate selfing (i.e., reduced herkogamy). Third, given the importance of the Mediterranean region as a glacial refugium, we constructed historical projections of the possible distribution of *N. dubius* under the Last Glacial Maximum (LGM) to assess the probability that contemporary peripheral populations formed part of the recent historical distribution of the study species.

Material and Methods

Study Species Distribution

To provide a precise distribution map and to select populations of *Narcissus dubius* for study, a geolocalized database of known population locations was compiled from four main sources: the *Silene* database of the Conservatoire Botanique National Méditerranéen de Porquerolles, France, with 401 locations; the *IPE* database of the Instituto Pirenaico de Ecología, Jaca, Spain, with 39 locations; the Biodiversidad Valenciana database of the Valence community, Spain, with 208 locations; and the *Anthos* online database (<http://www.anthos.es>), with

80 locations. We also gathered personal data for 85 locations in northern Catalonia and France (J. D. Thompson, and see "Acknowledgments"). Only data with a resolution of $<1 \text{ km}^2$ were used in our study, and the initial total of 813 occurrence points was carefully checked to remove duplicates, producing a final number of 363 occurrence points.

Narcissus dubius has a geographic range that extends from the Murcia province in southeastern Spain, where its distribution is limited by the Sistema Betica and Sistema Iberico mountains, to the southwestern corner of Provence in France (fig. 1). The species' distribution is fairly continuous along the east coast of Spain, with large numbers of populations in the coastal hills in the province of Valencia and up into Catalonia. However, three notable features form a contemporary and possibly historical break in population continuity and a basis for the identification of either central or peripheral populations. The first concerns the Ebro valley, where the Puertos de Tortosa-Beceite (northeast of the Sistema Iberico) creates a major barrier with high-elevation mountains. Populations that are located from Caspe to Tudela in Aragon province thus occur at the northwestern distribution limit in Spain. A second break in pop-

ulation continuity is associated with both the physical and the ecological barrier imposed by both the noncalcareous eastern tip of the Pyrenees and Albères mountains and the adjacent Roussillon coastal plain. Further north of this break, populations are spread in a lowland band around the Mediterranean to the southwestern corner of Provence, where the species reaches its distribution limits (fig. 1). Within this latter part of the distribution, there is a physical division across the delta of the Rhône valley (no populations are found there).

Based on these physical and ecological barriers, we distinguished four geographic groups of populations. In Spain, we distinguished central populations that occur in a continuous group of lowland populations close to the Mediterranean Sea (Catalonia, Valencia, and Alicante provinces) from those located in the more isolated inland part of the Ebro valley (Aragon) as peripheral populations. In France, central populations occur in a southwestern continuous group widespread across the lowland garrigues of Languedoc-Roussillon toward the Rhône valley. Peripheral populations are those that are present at the distribution limits of the species to the east and north of the extensive wetlands of the Camargue and at the confluence

Fig. 1 Distribution of the 363 locations of *Narcissus dubius* compiled from four different data sources. Diamonds represent populations where the ecological niche, floral measures, and morph ratio were quantified; squares represent sites used for floral measures and morph ratio estimates; open circles represent sites where only morph ratio estimates were made; and filled circles represent locations that were used only to model the climatic niche of this species.

of two major river valleys (the Gardon and the Rhône). The distinction of these central and peripheral groups of populations is thus based on contemporary physical and ecological breaks in the distribution that may have played an important role regarding population isolation in recent history.

Modeling Distribution Patterns in Relation to Past and Present Climate

To study climatic characteristics of populations, we extracted annual mean temperature and precipitation, temperature seasonality, and precipitation of the driest month for the 363 locations of *N. dubius* populations from the WorldClim database (<http://www.worldclim.org>) at a 2.5-arc-minute resolution (Hijmans et al. 2005). These four variables were chosen in order to summarize the climatic conditions occupied by this species and to limit any multicollinearity among the 19 bioclimatic variables available in the WorldClim database and the altitude.

We carried out principal component analysis (PCA) of the four variables to characterize the climatic niche for the 363 locations. The distribution of *N. dubius* was modeled using four commonly used algorithms implemented in the biomod2 package in R (Thuiller 2014): generalized additive model, generalized boosting model, random forest, and Maxent. These probabilistic models require information on species' absence (i.e., data that are rarely available), hence the need to assume a virtual or pseudoabsence, which can be generated by computer simulation. The number of points used, their method of selection, and the need to calculate mean values across simulations for such pseudoabsence are important to model accuracy (Chefaoui and Lobo 2008; Barbet-Massin et al. 2012). Following the recommendations of these authors, we randomly selected 1000 pseudoabsence points across the study area and repeated the modeling procedure 10 times with different subselections of pseudoabsences (Barbet-Massin et al. 2012). We calibrated the different models with 70% of the data and evaluated the results with the remaining 30%. We repeated this procedure five times to limit the impact of this random-splitting strategy. Model predictive accuracy was evaluated using the standard measures of the area under the receiver operating characteristic (ROC; Hanley and McNeil 1982) curve and the true skill statistics (TSS; Allouche et al. 2006).

In order to get historical insights, we established a broad estimate of what might have been the distribution of *N. dubius* at the LGM and how climatic suitability might have changed across the species' distribution since the LGM. Although the species and its genus clearly predate this period (Santos-Gally et al. 2013), we have focused attention on the LGM because it is recognized as an important event that has dramatically modified species ranges in the recent past. Although the impact of the LGM can be assumed to be less for Mediterranean taxa than for more temperate taxa, it may have caused complex patterns of isolation in diverse microrefugia (Diadema et al. 2005; Rodríguez-Sánchez et al. 2009; Patsiou et al. 2014) rather than simply a major north-south contraction (Rodríguez-Sánchez et al. 2009; Alba-Sánchez et al. 2010; Besnard et al. 2013).

We projected the probability of occurrence in space and time on 2.5-arc-minute grids representing the climatic condi-

tions at the current time and at the LGM (i.e., ~21,000 yr BP). These forecasts assume climate niche conservatism (Wiens and Graham 2005), for which there is now evidence (e.g., Martínez-Meyer and Peterson 2006). Past climate information was generated by the Palaeoclimate Modeling Intercomparison project (Braconnot et al. 2007) and was made available by WorldClim. We took into account the variability in the LGM climate projections by considering the output of two different global circulation models (MIROC3.2 and CCSM). According to Swets (1988) and the means of ROC and TSS scores obtained across our ensemble of models (mean ROC score: 0.911 ± 0.016 , mean TSS score: 0.719 ± 0.034), we can assume that our species distribution models performed well. In order to summarize these forecasts and obtain a single map for each time period, we computed mean values across the different projections, weighted by their respective model predictive accuracy (i.e., TSS scores).

Due to the absence of fossil data for this species, we were not able to validate our historical projections with empirical evidence. Our interpretations are thus made with caution.

Ecological Niche

The ecological niche was studied in terms of the broad vegetation type and the fine-scaled abiotic and biotic characteristics of patches where the species is abundant. This was done during peak flowering (March) in five randomly selected populations in each of the four geographical groups of populations (fig. 1). At each site, the range of broad habitat characteristics included the type of vegetation cover and the height of each biological type (Raunkiaer 1934) in the plant community (therophytes, hemicryptophytes, geophytes, chamephytes, and phanerophytes present in the phenological niche of the study species), slope, and elevation.

Fine-scaled ecological characteristics were studied in three quadrats (each 4 m²) that were established in high-density patches, at least 5 m apart, in each population. For each quadrat, a soil sample was collected, dried at 40°C for 48 h, sieved at 2 mm, and stored before analysis. Conductivity, pH, and salinity were measured using a Eutech Cyberscan. After mixing 10 g of dry soil with 20 mL of water, we blended the solution for 20 min, separated phases using a centrifuge (10 min), and measured values in the supernatant at room temperature (ca. 20°C). The total amounts of carbon (C) and nitrogen (N) were determined using a ThermoFinnigan Flash EA 1112 series on 70-μg samples grinded with a crusher (3 min, frequency = 30). Water-retention potential is the percentage of water lost after drying a wet soil for 48 h at 40°C. Water-retention capacity was then calculated as the percentage of water remaining in previously 40°C-dried soil by again drying the sample at 110°C for 5 h. Organic matter was estimated as the percentage of matter lost after burning a dried sample at 500°C for 5 h.

In each quadrat, 100 contact points were established with 20 × 20-cm divisions of the 4 m². We also estimated the mean height of each biological type. At each point, we recorded contact with one or more of the following elements: bedrock, blocks (>25 cm), stones (2.5–25 cm), gravel (0.5–2.5 cm), bare soil, lichen, moss, herbaceous litter, woody litter, and other plant species. Each contacted plant species was identified and attributed to a biological type (Raunkiaer 1934). We were inter-

ested in species only growing in the phenological niche of *N. dubius* and thus did not identify other species growing later in the spring. Preliminary observations of the data showed that bedrock, block, and stone had low values (making statistical analyses difficult for each parameter), and so we summed them at the quadrat level to make a composite substrate variable. When several components were touched at a given point, we constrained the value of each contact point to 1 so that the total cover per quadrat did not exceed 100. For each quadrat, we calculated species richness (the number of species touched during the contact point sampling) and the Shannon diversity index and its associated Hill number (Jost 2006). An important point here is that our aim is to identify the characteristics of the microniche where plants grow and when plants grow. This, of course, includes other species, which we detected in our study. We limit this to species present and growing only during the phenological cycle (niche) of *N. dubius*. These species are identified at the time of peak flowering of *N. dubius*. The many other species that may be present but in a different phenological niche are not quantified here.

All statistical analyses were performed using R 3.1.1 (R Development Core Team 2010), integrating central and peripheral population groups in each country. We fitted a linear mixed effect model (with country and geographic group as fixed effects and population as a random factor) and carried out two-way analysis of deviance based on type 2 Wald χ^2 tests for mixed models. We corrected *P* values following the Benjamini-Hochberg false discovery rate method (Benjamini and Hochberg 1995). We present *P* values that fall between 0.05 and the corrected value as weakly significant and those that are less than the corrected threshold as highly significant.

To quantify amounts of variation for each variable, we calculated the coefficient of variation between quadrats (within-population variation) and between populations (using mean values across quadrats) in each geographic group. Single-tailed Wilcoxon tests were carried out using the different traits to test for differences in within- and between-population variation among peripheral and central populations. Field studies and experimental laboratory analyses were conducted during spring 2013 and 2014.

Floral Polymorphism

Narcissus dubius flowers in February and March. Plants usually bear 3–5 flowers, but this can range from 1 to 12, depending on plant size and location (Worley et al. 2000). Flowers show a stigma-height dimorphism; individual plants are either long-styled (L-morph) or short-styled (S-morph), and both have anthers at roughly the same height in the flower (Baker et al. 2000a). This species is primarily pollinated by hawk moths, flies, and solitary bees (Baker et al. 2000a). Individuals are self-compatible and exhibit no intramorph incompatibility (Baker et al. 2000b).

In order to assess geographic variation in the presence of the two floral morphs, morph ratio counts were made throughout the range of the species in a total of 74 populations (fig. 1; app. A; apps. A–C available online). Population counts were made during spring 2013 or 2014. In each population, we aimed to sample at least 75–100 flowers to determine the morph ratio. Plants were sampled more than 2 m apart to avoid resampling

the same individual. If S-morph individuals were not found, sampling continued up to 200 flowers to check for an eventual very low frequency of S-morph individuals. The number of individuals was estimated visually to provide an indicator of population size in eight classes (with *n* = the number of individuals: $n < 10$; $10 < n < 50$; $50 < n < 100$; $100 < n < 500$; $500 < n < 1000$; $1000 < n < 5000$; $5000 < n < 10,000$; $10,000 < n$). In 19 populations, <75 flowers were sampled due to small population size.

To quantify variation in the relative position of sex organs, seven populations were selected at random in each of the four geographic groups (fig. 1). In 2014, in each population, we measured flower length, stigma height, and the upper and lower extremity of both upper and lower anthers on 30 flowers, each from a different L-morph individual (fig. 2). These were fully developed mature flowers. Flowers of the S-morph were not measured and included in the analysis, because they were uncommon or absent in many populations and do not show significant differences in flower size or anther position compared to the L-morph (Baker et al. 2000a). The only morph of interest to our question is the L-morph that occurs in dimorphic populations and alone in monomorphic populations.

First, we quantified the frequency of L-morph individuals for which the stigma is positioned above the upper anthers in each population. We then calculated stigma-anther separation, after correction for variation in flower size (following Baker et al. 2000a), as the distance from the top of the upper anther to the height of the stigma (fig. 2). This measure quantifies both

Fig. 2 Floral measures made on flowers of the long-styled morph of *Narcissus dubius*. All measures were made from the base of the ovule (O): corolla length (a), upper (b) and lower (d) extremity of the upper anther, stigma height (c), upper (e) and lower (f) extremity of the lower anther. Adapted from Arroyo and Dafni (1995).

Fig. 3 Principal component analysis of climate data for all known population locations (gray dots). The four climate variables are temperature seasonality (*a*), mean precipitation of the driest month (*b*), mean annual precipitation (*c*), and annual mean temperature (*d*). Ellipses were drawn with the ADE4 package to include 75% of each class variance. The gray ellipse represents all the locations, and the colored ellipses depict the position of the four geographical groups of populations. P = peripheral populations, C = central populations, F = France, S = Spain.

stigma-anther separation (positive values indicative of herkogamy when the stigma is above the anthers) and how deep the style is within the top anther whorl (zero or negative values). To test for variation in stigma-anther separation, we fitted two linear mixed effect models (with country and geographic group as fixed factors and population as a random factor) and performed two-way analysis of deviance based on type 2 Wald χ^2 tests (Nelder and Baker 1972). A spatial autocorrelation analysis was also performed (app. B) to verify that there was no major spatial correlation among populations that would otherwise hinder our interpretations of spatial patterns.

Results

Climate Analysis and Projected Past Distribution

The projection of the first two axes of the PCA covers 87% of the variation of the whole data set (fig. 3; table 1). The first component is driven primarily by two variables: the precipitation of the driest month and the mean annual temperature. The second component is primarily the result of temperature seasonality and, to a lesser extent, annual precipitation.

We used the *s.class* function from the ADE4 package to describe climatic variation among populations where the ecological niche was characterized. The ellipses drawn include 78%

of the data of the group. This allows us to identify the relative position of the four different population groups regarding the overall climatic niche of the species, whose center is represented by the intersection of the two axes (fig. 3). Central populations in France and Spain and peripheral populations in France occur in the same range of variation along axis 2. These three groups show a gradual decline in mean annual temperature and a higher mean precipitation of the driest month (i.e., a less marked Mediterranean climatic regime) toward peripheral and central populations in France, which differ from central popula-

Table 1

Results of the Principal Component (PC) Analysis

	PC-1	PC-2	PC-3	PC-4
Eigenvalues	2.6502	.8276	.2920	.2303
Projected inertia (%)	66.256	20.689	7.299	5.756
Pearson <i>r</i> :				
BIO1	.90	.13	.32	.28
BIO4	.72	.65	.025	.26
BIO12	.74	.61	.14	.25
BIO14	.89	.14	.41	.14

Note. BIO1 = mean annual temperature, BIO4 = temperature seasonality, BIO12 = mean annual precipitation, BIO14 = mean precipitation of the driest month.

tions in Spain (i.e., toward the left of axis 1). Peripheral populations in Spain are clearly distinct from all other populations (on axis 2) in terms of their higher seasonality: four of the five populations are beyond the gray circle representing the mean climatic niche of the species. This illustrates the more continental climatic regime of their location.

The current (fig. 4a) and historical (fig. 4b, 4c) projections of the probability of occurrence of *Narcissus dubius* populations based on current climate data and potential climate of the LGM were obtained using two simulation models (CCSM and MIROC). Differences in past climate predictions do not qualitatively change the results. In the area where central populations occur in Spain, large areas of suitable climate may have existed during the LGM. This was not the case for the area where peripheral populations now occur in Spain. In France, suitable potential climate probably existed as a belt spread above actual sea level around the Mediterranean coastal belt (fig. 1). For current emergent land, only two disjunct areas were climatically suitable for this species, one in the area occupied by peripheral populations north of Marseille and the other southwest of Narbonne in part of the area occupied by central populations.

Microecological Niche

Central and peripheral populations of *N. dubius* showed differences in the mean value of several abiotic and biotic components of the ecological niche (table 2). For the abiotic niche, the composite variable of substrate elements (combination of bedrock, blocks, and stones) showed a significantly lower cover in peripheral populations (fig. 5a), the cover of bare soil was

significantly higher in peripheral populations (fig. 5b), and the C/N ratio (fig. 5c) was significantly lower in peripheral populations only in Spain. In general, soil properties varied significantly only between countries (table 2), all showing lower values in Spain, except for pH that was higher in Spain than in France.

For the biotic niche, we found that the cover of therophytes was significantly higher in peripheral populations (fig. 5d). The number of species per quadrat (fig. 5e) and the Hill's number associated with the Shannon diversity index (Jost 2006; fig. 5f) both showed a similar trend of increase in peripheral populations, although this trend was not statistically significant.

Ecological niche variation among quadrats (fig. 6a) showed no significant difference between central and peripheral populations (Wilcoxon test, $V = 383$, $P = 0.2184$), while variation among peripheral populations was significantly greater than that among central populations (Wilcoxon test, $V = 204$, $P = 0.0218$; fig. 6b).

Floral Polymorphism

A total of 74 populations were sampled to assess morph ratio variation (fig. 7). In the central group of populations in the Languedoc-Roussillon region of southern France ($n = 30$ populations, for a total number of 3753 individuals), *N. dubius* populations are in majority dimorphic but with a consistently L-morph-biased ratio (80%–95%). Only three very small populations ($n = 26$, 26, and 66 flowering plants) were monomorphic, which is thus potentially an artifact of low sample size. In the peripheral group of populations, only the L-morph was observed in the 20 studied populations (3655

Fig. 4 Modeled probability of occurrence of *Narcissus dubius*: contemporary distribution (a) and at the Last Glacial Maximum according to CCSM (b) and MIROC (c) climate models. For the two historical projections, historical sea level is drawn as a solid line, while the contemporary coastline is represented by a dotted line. The color scale represents the probability of climate suitability.

Table 2

χ^2 Values and Significance Tests for the Analysis of Deviance of Abiotic and Biotic Components of the Ecological Niche of *Narcissus dubius* in Central (C) and Peripheral (P) Populations in Spain and Southern France

Niche component	Country	C vs. P	Interaction
Soil analyses:			
pH	10.4324**	.4786	2.2314
Soil conductivity	6.5203**	1.804	1.7691
Water-retention potential	10**	1.1525	3.8906
Water-retention capacity	16.8847**	2.7819	1.1523
Organic matter	12.351**	1.1014	3.119
Carbon	12.233**	.3362	1.2719
Nitrogen	3.8662**	.3119	.1619
Carbon/nitrogen	7.1131**	8.0987**	15.0746**
Point contact cover data:			
Mineral	1.0463	3.8934*	.8522
Gravel	4.3522	.4529	.1567
Bare soil	2.3715	5.5124*	.604
Litter	.6645	.3561	1.4115
Therophytes	.0201	10.6814**	.505
Hemicryptophytes	.27	.1146	.1401
Geophytes	.2151	.7177	0
Chamephytes	2.4277	.1384	.0376
Phanerophytes	.0754	.4699	.4021
Species data:			
Species no.	.048	2.722	.3583
Shannon diversity index	.1008	3.1875	.2525

Note. Mineral cover is a combination of bedrock, blocks, and stones. All tests are based on a single degree of freedom.

* $0.05 > P > \text{corrected significance level}$.

** $P < \text{corrected significance level}$.

individuals). In Spain, a similar pattern was observed: 10 of the 14 central populations in the coastal hills (a total number of 1703 sampled individuals) were dimorphic, with an L-morph-biased ratio (75%–99%). In contrast, seven of the nine peripheral populations were monomorphic for the L-morph. In the two dimorphic peripheral populations, only a single S-morph individual (in a total of 45 and 315 studied plants per population, respectively) was observed per population. Hence, in this peripheral group of populations, only two S-morph plants were observed in a total of 1944 sampled plants (fig. 7). There was no significant correlation between population size and morph ratio in dimorphic populations ($R^2 = 0.014$, $P > 0.05$).

Floral traits of the L-morph showed several patterns of variation (fig. 8; table 3) that accompany differences in the occurrence of dimorphic and monomorphic populations. The measures we made were standardized as described above to remove effects of correlated variation among traits (app. C). Corolla size showed significant differences among countries ($P < 0.001$; fig. 8a), with smaller flowers in France than in Spain and a significant country-by-geographical-group interaction ($P < 0.01$). Spanish peripheral populations had significantly larger flowers than central populations ($P < 0.001$), whereas French peripheral populations had significantly smaller flowers than central populations ($P < 0.001$). The frequency of individuals with stigmas above the anthers (fig. 8b) was significantly higher ($P = 0.031$) in both groups of central populations

(19%) compared to in peripheral populations (8% and 10.5% for French and Spanish peripheral populations, respectively). Stigma-anther separation (fig. 8c) showed a similar trend, with less stigma-anther separation in peripheral populations ($P = 0.002$), particularly peripheral populations in France (fig. 8c).

Discussion

This study provides an illustration of how the ecological niche and traits associated with a floral polymorphism vary among different parts of the range of a Mediterranean geophyte, with consistent patterns of change toward geographically peripheral populations. Climate-based historic projections of distribution changes associated with the LGM provide a historical context for the interpretation of these contemporary patterns.

Ecological Niche Variation

The ecological characteristics of the habitat occupied by populations of *Narcissus dubius* show a repeated pattern of variation between central and peripheral populations. In terms of the microecological niche (i.e., the precise ecological

Fig. 5 Mean values per quadrat of abiotic and biotic features of the ecological niche of *Narcissus dubius* that show significant variation among central and peripheral populations. a, Percentage cover of mineral elements. b, Percentage cover of bare soil. c, Carbon/nitrogen ratio. d, Percentage cover of therophytes. e, Number of species. f, Hill number associated with the Shannon diversity index.

Fig. 6 Ecological niche variability of central and peripheral populations of *Narcissus dubius* among quadrats within populations (a) and among populations (b). Squares represent soil variables, triangles represent biotic count variables, and circles represent abiotic count variables. Values on the two axes are standardized values of the coefficient of variation. For a point that falls on the bisector, trait variability is identical. Points to the left of the bisector signify greater variability among peripheral populations; points to the right of the bisector signify greater variability among central populations.

conditions where plants grow), peripheral populations occur in patches with a higher cover of bare soil and therophytes. Peripheral populations of *N. dubius* thus tend to occur in richer and more diverse vegetation, as therophyte communities of the Mediterranean flora are very diverse (Madon and Médail 1997; Blondel et al. 2010). In addition, peripheral populations in Aragon (Spanish periphery) have a very different macroniche in terms of broad habitat characteristics; they occur in Mediterranean steppic grasslands that are very different from the rocky calcareous garrigues where this species occurs across the Mediterranean coastal belt of eastern Spain and southern France, especially regarding soils that are poorer in organic matter (and thus total carbon) and have lower water-retention potential. These peripheral populations in Spain also occur in a more continental type of climatic regime.

Other studies have shown variable results in terms of the habitat characteristics of species in peripheral populations, with richer and more diverse vegetation in some (Jump and Woodward 2003) but not all (Lönn and Prentice 2002; Murphy et al. 2006; Mueller et al. 2011) cases. Variation in habitat quality also does not show a clear repeatable pattern of differences in studies of central and peripheral plant populations (Medail et al. 2002; Duffy et al. 2009). In our case, the consistent changes in the microecological niche (i.e., more open habitats with a greater cover of annual plants in peripheral populations) might represent a shift toward less competitive and more disturbed ecological conditions at range limits (Grime 1988; Madon and Médail 1997). Verification of this hypothesis will require experimental investigation of the impact of ecological conditions on individual fitness and whether local adaptation and/or phenotypic plasticity are associated with ecological niche variation in different parts of the species' range (Holt and Keitt 2004; Pearman et al. 2008a).

Finally, in the comparison of ecological trait variation, interpopulation variability is higher among peripheral populations than among central populations. This is not an artifact of having sampled populations over greater distances for peripheral populations. The mean ($\pm$ SE) distance among studied peripheral populations is 83 km ($\pm$ 13.0) in France and 56 km ($\pm$ 6.2) in Spain, and the mean distance among studied central populations is 70 km ($\pm$ 11.8) in France and 145 km ($\pm$ 25.1) in Spain. In fact, in Spain, the central populations we studied are farther apart than peripheral populations. Our results, thus, differ from findings in other studies that report a narrower, more specialized ecological niche in peripheral populations (Kavanagh and Kellman 1986; Svensson 1992; Rivero-Guerra 2008), although comparative analyses of several taxa have found little support for this trend (Leuschner et al. 2009; Wasof et al. 2013). In our study, the consistent ecological changes in populations in two distinct peripheral areas of the distribution of *N. dubius* provide evidence that niche shift in peripheral populations is not necessarily linked to reduced niche width and specialization.

Loss of the Floral Polymorphism in Peripheral Populations

Narcissus dubius has a mating system in which both selfing and outcrossing occur (Baker et al. 2000a) and in which outcrossing can involve assortative (within-morph) and disortative (between-morph) mating. A strong L-biased morph ratio is commonly observed in floral polymorphic *Narcissus* species (Dulberger 1964; Arroyo and Dafni 1995; Arroyo et al. 2002; Thompson et al. 2012). This pattern is consistently observed in dimorphic populations across the range of *N. dubius*, for which the highest frequency of the S-morph does not exceed 25% of a population. We found no associa-

Fig. 7 The distribution of monomorphic (filled circles) and dimorphic (open circles) populations of *Narcissus dubius* across its distribution in the western Mediterranean. The two open circles with adjacent asterisks are peripheral populations in Spain with a single S-morph individual.

tion between population size and morph ratio, which is consistent with previous results (Baker et al. 2000a). As shown by theoretical models (Baker et al. 2000b), a strong L-biased morph ratio in natural populations is likely to be due to a higher rate of assortative mating in the L-morph compared to in the S-morph. In *N. dubius*, this may be favored by the absence of reciprocity between anther position in the L-morph and stigma height of the S-morph, the self-compatibility of this species that allows for selfing (Baker et al. 2000a), and the reduced herkogamy of the L-morph that we report in monomorphic populations. Indeed, peripheral populations of *N. dubius* are almost exclusively monomorphic, lacking the S-morph. Occasionally, populations lack the S-morph in the central part of the range in both Spain and France. Such monomorphic central populations are either very small population patches (<50 individuals) or have incurred recent disturbance (fire).

Mechanisms that favor outcrossing can be heterogeneously distributed across the range of widespread species (Barrett

2001; Cheptou 2011), and as a result, many polymorphic species show changes in the relative frequency of morphs in different parts of their range (Weller 1986; Barrett and Richards 1990; Arroyo and Dafni 1995; Arroyo et al. 2002; Barrett et al. 2004; Thompson et al. 2012), sometimes with the loss of particular morphs (Shore and Barrett 1985; Barrett et al. 1989; Eckert et al. 1996; Hodgins and Barrett 2008). In *Narcissus papyraceus* in southern Spain, northern peripheral populations show a loss of the S-morph (Arroyo et al. 2002) and lower levels of genetic diversity than central populations (Simón-Porcar et al. 2015). The latter author found a significant correlation between genetic diversity and the frequency of the S-morph, which further confirms a trend toward reduced outcrossing in peripheral populations. This follows the central-peripheral model that predicts lower genetic diversity at the edge of the distribution (Eckert et al. 2008; Sexton et al. 2009). Unlike in *N. papyraceus*, where peripheral populations are often smaller than central populations (Arroyo et al. 2002), the monomorphic peripheral populations of *N. dubius* are mostly large

Fig. 8 Floral biology of the long-styled morph of *Narcissus dubius* in central (C) and peripheral (P) populations in France and Spain. *a*, Mean corolla size. *b*, Percentage of individuals with a stigma positioned above the anthers. *c*, Mean stigma-anther distance (i.e., the distance of the stigma to the upper tip of the upper anther).

and fairly abundant within the region where they occur, particularly in Provence. This occurrence of monomorphic populations could be due to selection that favors assortative mating and/or selfing or be a consequence of stochastic forces acting in peripheral populations.

The loss of the S-morph and reduced stigma-anther separation in peripheral populations may be linked to pollinator-induced selection that enhances assortative mating and/or self-fertilization (Belaoussoff and Shore 1995; Baker et al. 2000b; Herlihy and Eckert 2004). Indeed, reduced herkogamy may permit reproductive assurance in the absence of pollinators (Lloyd and Schoen 1992; Affre and Thompson

1999; Moeller 2006). Changes in pollinators and reduced pollination service have been documented in other Mediterranean *Narcissus* species in relation to changes in morph abundance, particularly a relative loss of long-tongued pollinators that assure the reproduction of the S-morph (Arroyo and Dafni 1995; Pérez-Barrales and Arroyo 2010; Santos-Gally et al. 2013). *Narcissus dubius* is visited by a range of generalist pollinators in southern France (J. D. Thompson, personal observations), including long-tongued Lepidoptera, primarily *Macroglossum stellatarum*, and short- and long-tongued bees (mostly *Apis* and *Anthophora*). We have no evidence for any variation in composition among central and peripheral populations, and because the widespread distribution of the long-tongued pollinators covers the peripheral group of populations in France, it is therefore unlikely that the loss of the long-tongued pollinators causes a pollinator-based shift toward increased assortative mating and selection for selfing in peripheral populations. This will, however, require further investigation for confirmation.

In floral polymorphic species, morph ratio variation has also been found to be linked to the spatial and temporal dynamics of populations, which may lead to modifications in the genetic assemblage of colonizing populations and randomly cause a decline in the abundance or loss of particular morphs (Barrett et al. 1989; Eckert and Barrett 1992; Husband and Barrett 1992; Hodgins and Barrett 2008; R. Berjano, P. Gauthier, V. Pons, and J. D. Thompson, unpublished manuscript). The loss of the S-morph in peripheral populations of our study species could thus also result from stochastic events associated with historical processes of range contraction and spread.

Insights from the Recent History of N. dubius

Our historical projections of the distribution of *N. dubius* at the LGM provide insights into this potential role of stochastic variation during periods of range contraction and expansion of *N. dubius* populations. Although this timescale does not allow us to fully cover the species' history due to its probable ancient origin (Santos-Gally et al. 2012), the LGM has been found to be a major cause of contemporary

Table 3

χ^2 Values and Significance Tests for the Analysis of Deviance of Corolla Size and Relative Stigma-Anther Separation and ANOVA Table for Frequency of Individuals with Stigma above the Anthers for the Long-Styled Morph of *Narcissus dubius* in Central (C) and Peripheral (P) Populations in Spain and Southern France

Flower measure	Country	C vs. P	Interaction
Analysis of deviance table:			
Corolla size	27.5721*	1.2298	8.6410*
Relative stigma-anther separation	2.4718	9.3771*	2.0394
ANOVA table:			
Frequency (F) of individuals with stigmas above the anthers	.4742	5.2689*	.0132

Note. All tests involve a single degree of freedom.

* $P < \text{corrected significance level}$.

patterns in many species (Hewitt 1999; Rodríguez-Sánchez et al. 2009; Alba-Sánchez et al. 2010). Nevertheless, we interpret historical projections with prudence, given the absence of palaeoecological and phylogeographical data for this taxon (Gavin et al. 2014) and the fact that nonanalogous climatic conditions between the current time and the LGM may influence the results (Pearman et al. 2008b; Veloz et al. 2012).

Suitable climatic conditions for the persistence of *N. dubius* during the LGM probably existed in the coastal hills of south-east Spain (<1000-m elevation). This area has been identified as a potential LGM refugia for other Mediterranean plants (Carrión et al. 2003; Médail and Diadema 2009) and, during previous Pleistocene glaciations, for oceanic (Taberlet et al. 1998; Médail and Diadema 2009) and Mediterranean (Lumaret et al. 2002; Gómez and Lunt 2007; López de Heredia et al. 2007; Besnard et al. 2013) temperate species. At the LGM, the Ebro valley (current peripheral populations) appears to have been unsuitable for *N. dubius*. This area might have served as a refugium for temperate taxa, such as *Corylus avellana* L., now present only in a few shallow gorges in the Ebro valley (González-Sampériz et al. 2004; Valero-Garcés et al. 2004), but not for more typical Mediterranean taxa, such as our study species (González-Sampériz et al. 2008).

Since the LGM, an arid steppe habitat with a rather novel vegetation type has developed in this area (Pérez-Collazos et al. 2009). *Narcissus dubius* may thus have spread from southeastern refugia to colonize the Ebro valley since the LGM thanks to an adaptation to a more seasonal, continental-like climate. This colonization pathway has also been proposed for *Ferula loscosii* (Lange) Willk. (Pérez-Collazos et al. 2009), a species in which genetic homogeneity has been interpreted as a signal of recently founded populations. This interpretation may thus be applied to the almost complete loss of the S-morph in peripheral populations in this area. This loss may have occurred as a result of repeated founder effects and stochastic loss during colonization events or perhaps historical selection by pollinators. Given the already-low frequency of the S-morph in central populations, this loss may indeed occur fairly easily and thus repeatedly. An interesting point here is that *N. dubius* occurs in a clade with monomorphic species (Santos-Gally et al. 2013).

In southern France, it is not clear exactly where elements of the Mediterranean flora persisted during the LGM (Pons and Vernet 1971); the precise location of potential refugia is difficult, and hypotheses for their location should be interpreted cautiously. For *N. dubius*, LGM projection of potentially suitable climate (fig. 4b, 4c) occurred in an area that is now either submerged or on and around the calcareous mountains of southwestern Provence and in the Languedoc-Roussillon region near Narbonne, where several low-elevation calcareous ranges were islands in recent history. These two areas have been historically isolated by a large barrier of unsuitable sandy and coastal areas and have several restricted endemic species (Fréville et al. 2004; Pouget et al. 2013) or disjunct en-

demic species whose populations are peripheral isolates of more southern Mediterranean species (sensu Thompson 1999, 2005; G. Papuga, unpublished data).

Hence, although the historical projections and current patterns for peripheral populations are less clear in southern France than in Spain, the possibility of persistence in different microrefugia in the coastal belt of southern France cannot be ruled out. Elsewhere, it has been proposed that, despite a globally unfavorable climate matrix, the Mediterranean Basin probably contained several microrefugia (Felinier 2011), even locally (Patsiou et al. 2014). Given the complete absence of the S-morph from all the contemporary peripheral populations (despite their size and abundance), their reduced flower size, and their reduced stigma-anther separation, it is possible that the S-morph might have been lost as a result of a bottleneck or selection during repeated range shifts (this species may disperse quite well; Santos-Gally et al. 2012) from a local refugium. As a result, the southern part of the Rhône valley (fig. 7, inset) may in fact represent an admixture zone where two colonization fronts have met, as observed for genetic diversity in *Quercus ilex* L. (Lumaret et al. 2002), and not only a simple separation between contemporary central and peripheral populations.

Conclusion

This comparison of the ecological niche and floral polymorphism in the different parts of the range of *N. dubius* in the western Mediterranean illustrates how diverse directions of colonization out of distinct microrefugia may have shaped contemporary patterns of variation. In eastern Spain, colonization toward Aragon has involved a shift in ecological conditions and an almost complete loss of the S-morph. In Mediterranean France, trait variation could reflect persistence and spread from different refugia and not a gradual shift from central to peripheral populations. We have now investigated a collaborative phylogeographic study of this species in order to test these possible interpretations for the historical evolution of contemporary patterns of ecological and genetic variation.

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