Heredity (2008) 100, 275–285

www.nature.com/hdy

ORIGINAL ARTICLE

Adding a third dimension to the edge of a species' range: altitude and genetic structuring in mountainous landscapes

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In addition to the topographical and ecological barriers, other landscape features may also subtly influence the patterns of gene flow and spatial genetic structuring at species' borders. This paper focuses on the role played by altitudinal gradients that characterize mountainous landscapes. We formulate and test the hypothesis that when the distribution boundaries of plant species intersect mountainous landscapes, altitudinal gradients in ecological conditions may considerably enhance population subdivision and genetic structuring at the regional level. Using amplified fragment length polymorphism markers, we studied genetic diversity and differentiation in a set of 21 peripheral populations of the evergreen shrub *Lavandula latifolia* Med. (Labiatae) at its southernmost distribution limit in the Betic mountain ranges of southern Spain. Population size and abundance, and within-population genetic diversity, varied predictably with altitude, being highest at middle elevations and declining steadily towards both the upper and lower altitudinal distribution margins. Genetic differentiation tended to follow the opposite trend. These altitudinal patterns result from variation with elevation in the relative influence of gene flow and drift on the distribution of genetic variation. Genetic drift prevails around the upper and lower altitudinal limits, whereas a situation closer to a drift-gene flow equilibrium exists at the center of the altitudinal distribution. Altitudinal variation in the relative influences of gene flow and drift appears as an essential element in the interpretation of regional genetic structuring of *L. latifolia* at its mountainous distribution edge, and a factor which may influence the evolutionary potential of peripheral populations and the likelihood of local adaptation.
Heredity (2008) **100,** 275–285; doi:10.1038/sj.hdy.6801072; published online 17 October 2007

Keywords: altitudinal gradients; genetic differentiation; genetic diversity; *Lavandula latifolia*; population size; species range limits

Introduction

The opportunities for genetic differentiation, local adaptation and/or speciation may be either enhanced or reduced at the limits of species' geographical ranges, depending on the magnitude and directionality of gene flow. In species with relatively sharp range boundaries and facing no major impediments to gene flow, asymmetrical gene flow from a populous center may limit or preclude adaptation of sparser populations at the periphery, even if the latter locally experience intense directional selection (García-Ramos and Kirkpatrick, 1997; Kirkpatrick and Barton, 1997). In contrast, when populations on the distribution edge become isolated from gene flow from the central area, genetic drift in combination with responses to local selection pressures can promote genetic divergence and rapid evolution (García-Ramos and Kirkpatrick, 1997). The pattern and magnitude of gene flow in the neighborhood of a species' distribution edge thus become critical determinants of genetic structuring and, ultimately, of the evolutionary prospects of peripheral populations (Lenormand, 2002; Alleaume-Benharira *et al*., 2006).

Topographical (e.g., rivers, mountain ridges) or ecological (e.g., unsuitable habitats) limitations to gene flow, acting in the vicinity of range edges, are the most obvious candidates influencing the evolutionary fate of peripheral populations, particularly in the case of organisms endowed with little mobility like plants. Comparisons between peripheral and central populations of plants have often shown increased genetic differentiation, and reduced within-population and overall genetic diversity of peripheral isolates relative to populations at central positions in the distribution range (Lammi *et al*., 1999; Lönn and Prentice, 2002; Eckstein *et al*., 2006). In addition to topographical and ecological barriers, however, other landscape features may also subtly influence patterns of gene flow and spatial genetic structuring at species' borders. In this paper, we will focus on some genetic consequences of the altitudinal gradients in ecological factors that characterize mountainous landscapes. These gradients may involve, among other factors, variation in rainfall, temperature, biotic interactions and plant community composition, diversity and dynamics (e.g., Beals, 1969; Cruden, 1972; Kalin Arroyo *et al*., 1982; Barclay and Crawford 1984; Schuster *et al*., 1989), whose altitudinal variation may influence habitat suitability for a given species. Specifically, we will formulate and test the hypothesis that when the distribution boundaries of

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Received 4 May 2007; revised 31 August 2007; accepted 24 September 2007; published online 17 October 2007

plant species intersect mountainous landscapes, altitudinal ecological gradients may contribute to the enhancement of regional population subdivision and genetic structuring.

In mountainous landscapes, individual plant species are ordinarily confined to distinct altitudinal ranges. Abundance typically peaks at some intermediate altitude where environmental conditions are optimal for the species, and declines towards upper and lower altitudinal limits as a consequence of deteriorating habitat suitability (Beals, 1969). Populations at the upper and lower distributional limits occupy ecologically marginal conditions and should be considered as peripheral populations on the altitudinal axis of distribution of the species. As already noted by Darwin (1859) (pp 174–175), variation in abundance with changing altitude is just a particular case of an 'abundant center distribution' (ACD) pattern in which the abundance of individuals within a population and the density of populations within an area generally decline from the core to the periphery of a species' geographical distribution range (Hengeveld and Haeck, 1982; Brown, 1984; but see the review by Sagarin and Gaines, 2002 for exceptions). Variations in the drift-gene flow balance along the altitudinal dimension of a species' distribution are expected to originate altitudinal variation in genetic structuring and diversity of populations, particularly at those located at the upper and lower distributional limits. Consistent with this expectation is the observation that genetic structuring along altitudinal gradients may vary from weak (Neale and Adams, 1985; Schuster *et al*., 1989) to moderate-high (Premoli, 2003; Reisch *et al*., 2005). In mountainous landscapes, altitudinal ACD-like patterns may originate a distinct component of genetic variation due to the variable balance between drift and gene flow along the altitudinal axis, essentially similar to that ordinarily considered by genetic models of evolution at the edge of geographical ranges. Such an effect may occur anywhere within the geographical range of a species, but it should be most apparent and evolutionarily relevant on geographical distribution borders, where smaller and sparser populations already living near the environmental limits for the species will be particularly susceptible to further ecological deterioration at the upper and lower altitudinal limits. We thus hypothesize that when the geographical limits of plant species intersect mountainous landscapes, the component of genetic structuring arising from altitudinal ecological gradients will contribute to enhance regional population subdivision and genetic differentiation.

This paper examines the preceding hypothesis by analyzing the genetic structure and genetic diversity of peripheral populations of the evergreen shrub *L. latifolia* Med. at its southern Spanish distribution limit, using amplified fragment length polymorphism (AFLP) markers. The following questions are specifically addressed: (1) what is the relative importance of drift and gene flow in determining genetic structuring and diversity of populations of *L. latifolia* at the edge of its geographical range? (2) Do genetic structuring and genetic diversity depend on location along the species' altitudinal range? and (3) To what extent does such altitudinal variation contribute to genetic structuring at a regional scale?

Materials and methods

Study plant

L. latifolia is an evergreen shrub occurring in the eastern Iberian Peninsula and southern France (Figure 1a). In our study region (see below), the species typically occupies well-insolated habitats at middle elevations, such as the understory of open pine or oak forests, forest clearings and edges, and successional scrub. Individual plants live for up to 25–35 years. Local populations may persist for many generations on permanently favorable places like open forests and sparse scrublands on rocky outcrops or poor soils. Other habitats, like natural forest clearings on more humid locations with fertile soils, may remain favorable for the species for only a few generations. At these sites, *L. latifolia* populations decline to local extinction in a few decades after colonization, as succession proceeds and a developing forest canopy reduces insolation. Incidental observations suggest that populations at upper and lower altitudes may have higher average turnover rates than populations at middle elevations (CM Herrera, unpublished data).

Flowers are hermaphroditic and self-compatible, but $<4\%$ of flowers set fruit in the absence of pollinators. In the study region, the species is pollinated by a diverse array of bee, fly, and butterfly species (Herrera *et al*., 2006

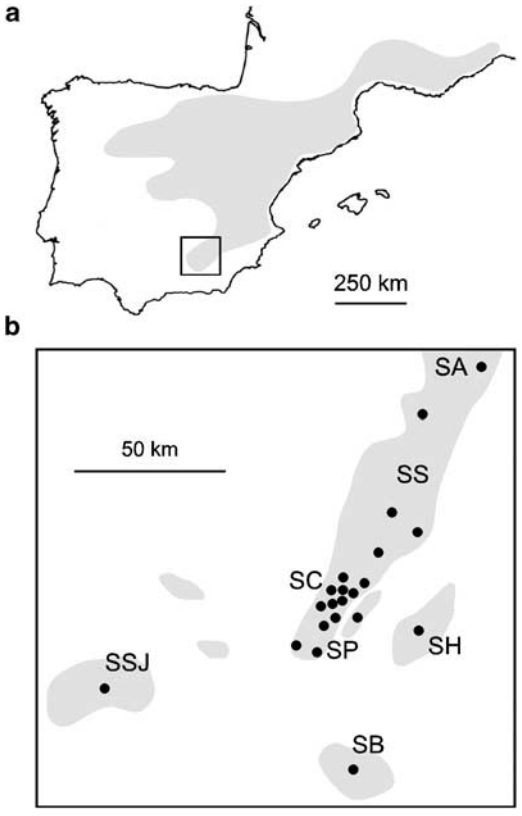

Figure 1 **(a)** Geographical distribution of *Lavandula latifolia* (shaded area). **(b)** Details of the distribution on the southernmost edge of the range and location of the 21 populations sampled for this study (dots). Based on Herrera *et al*. (2006), specimens in the University of Jaén herbarium (C Fernández-López, personal communication), and CM Herrera (unpublished data). The location of the seven mountain ranges sampled for this study is shown, coded as follows: SA, Alcaraz; SS, Segura; SC, Cazorla, SP, El Pozo; SB, Baza; SH, Huéscar; and SSJ, Sur de Jaén.

and references therein). Flowering takes place between early July and mid September, irrespective of elevation. Seeds lack special dispersal mechanisms, falling beneath or very close to the parent plant shortly after maturation (Herrera, 1987), although large herbivorous mammals might occasionally disperse seeds at long distances (Sánchez and Peco, 2002). Seeds are short-lived, becoming inviable after 2–3 years in the soil, hence the establishment of new populations will generally depend on seed colonization rather than regeneration from a pre-existing soil seed bank.

Study sites and methods

For this study, we sampled 21 *L. latifolia* populations on the southernmost edge of the species' distribution (Figure 1b), which intersects several major mountain ranges. Populations were located at all the major mountain ranges ('sierras') of the region where the species is known to occur: Sierra de Alcaraz (one population), Sierra de Segura (three), Sierra de Cazorla (ten), Sierra del Pozo (four), Sierra de Huéscar (one), Sierra de Baza (one), and Sierra Sur de Jaén (one). We are confident that all the southern peripheral areas where the species is relatively frequent were sampled. Altitude of sampling sites ranged between 990 and 1540 m asl, which encompasses the entire altitudinal range of the species in the region. Pairwise geographical distances between populations ranged between 0.25 and 170 km. Ideally, all populations should have been sampled over a small geographical area, so that potentially influential factors other than altitude were kept as constant as possible. Our sampling, however, was dictated by the actual distribution pattern of *L. latifolia* in its distribution border, which is characterized by wide gaps between isolated populations or groups of populations (Figure 1b). The size of the populations sampled was scored according to the following five-step scale: <50, 50–250, 250–500, 500–1000, and >1000 reproductive individuals. Three groups of populations were distinguished for the analyses of altitudinal variation, closely corresponding to the lower (950–1150 m asl), middle (1150–1300 m asl) and upper (1300–1550 m asl) thirds of the species' altitudinal range in the region (referred to hereafter as 'low', 'middle' and 'high' populations). Cutpoints used to define these groups were chosen so that populations were evenly distributed among altitudinal categories.

To obtain information on the size structure of populations and verify the hypothesized relationship between abundance and altitude under the ACD model, an extensive survey was conducted in 2006 in Sierra de Cazorla and Sierra del Pozo, where *L. latifolia* is most frequent, and natural vegetation has experienced the least anthropogenic disturbances in recent times. An area of about 60 km^2 within the Guadahornillos-Navahondona Nature Reserve was thoroughly surveyed by walking or driving. The altitude of the surveyed area ranged between 700 and 1750 m asl. The size of each population found was scored using the five-step scale described above, and altitude at its center was recorded using a GPS receiver.

Fresh leaf material was collected from 15 adult plants at each of the 21 sampling sites in spring–summer 2005. Widely spaced individuals were haphazardly selected from the whole area occupied by the population to minimize possible bias for genetic substructuring. Leaves were placed in small paper envelopes and dried immediately at ambient temperature in sealed containers with abundant silica gel. Before DNA extraction, dried leaf material was homogenized to a fine powder using a Retsch MM 200 mill. Total genomic DNA was extracted from approximately 35 mg of ground leaf material using DNeasy Plant Mini Kit (Qiagen, GmbH, D-40724 Hilden, Germany) by following the manufacturer protocol. DNA concentration of the extracts was estimated by running electrophoreses of 5 µl aliquots on 0.8% agarose gels. The AFLP analysis was performed essentially as originally described by Vos *et al*. (1995), with modifications involving the use of fluorescent dye-labeled selective primers following Applied Biosystems (2005). Restriction-ligation was conducted using *Eco*RI/*Mse*I endonuclease mixture and double-stranded adaptors. A total of 24 *Eco*RI + 3 *Mse*I + 3 primer pairs were first screened for selective amplification in a pilot study conducted on a random subsample of 12 individuals from four widely scattered populations. The following three primer pairs were finally chosen because of their higher polymorphism frequency: *Eco*RI-AGG/*Mse*I-CAT, *Eco*RI-AGG/*Mse*I-CGT and *Eco*RI-AGG/*Mse*I-CTG. Each individual plant sampled was fingerprinted using these three combinations. Fragment separation and detection were carried out using an ABI PRISM 3100 DNA sequencer. We used GeneMapper 3.7 software to score the presence or absence of each marker in each individual plant. To determine the reproducibility of the AFLP patterns, the whole process from restriction-ligation to final fingerprinting was repeated twice for a set of four samples at the beginning of the study. AFLP patterns from replicated samples were identical for the three primer combinations used.

Data analyses

In accordance with theoretical models (e.g., Nei and Roychoudhury, 1974; Nei, 1978), recent simulation studies of AFLP data show that standard errors of genetic distance and gene diversity estimates exhibit a decelerating decrease with increasing sample sizes and number of markers (Medina *et al*., 2006; Singh *et al*., 2006). As would be predicted from the results of these simulations, the combination of ≈ 200 polymorphic markers and 15 individuals per population used in this study was sufficient to produce reasonably narrow standard errors and adequate statistical power to detect differentiation among populations in *L. latifolia*, a species characterized by moderate to high genetic variability.

Prior to statistical analyses, markers that were monomorphic for the entire data-set were excluded, and the AFLP data matrix was tested for fragment size homoplasy by running correlations between size and frequency of AFLP fragments for successively narrower fragment size ranges, using the software AFLPSURV (Vekemans *et al*., 2002). Negative, statistically significant ($P<0.05$) correlations between size and frequency of AFLP fragments suggestive of size homoplasy were found for fragments in the 50–500 and 100–500 bp ranges. The size–frequency correlation disappeared ($r=-0.013$, $P=0.85$) after exclusively considering fragments between 150 and 500 bp, and excluding those which were present in <1 or $>99\%$ of individuals. All the analyses reported in this paper are based on this reduced subset of

$N = 207$ AFLP markers. All the individuals examined exhibited unique AFLP profiles.

The Bayesian procedures of Holsinger *et al.* (2002) and Holsinger and Wallace (2004) were used to analyze genetic structure and diversity, as implemented in the program Hickory version 1.0.4. These methods overcome some of the limitations of dominant markers to estimate genetic parameters (e.g., Lynch and Milligan, 1994; Zhivotovsky, 1999), and allow estimating F_{ST} from dominant marker data without prior information on the level of within-population inbreeding (F_{IS}) and without assuming Hardy–Weinberg equilibrium of genotypes (for details, see Holsinger *et al*., 2002). Analyses were conducted for all populations, and separately for populations located at the lower, middle and upper altitudinal belts. The following models were tested in each case: (i) a full model with noninformative priors for f (an estimate of F_{IS}) and θ^B (an estimate of F_{ST} under a random-effects model of population sampling), (ii) a model with $f = 0$, and (iii) a model with $\theta^B = 0$. Biologically unrealistic estimates of f may sometimes be obtained for dominant marker data with the Bayesian method implemented in the program Hickory (Holsinger and Lewis, 2003). This was the case with our data (see Results), so we also ran (iv) a free model where θ^B is estimated without estimating f. In this free model, f values are chosen at random from its prior distribution, and the θ^B estimates obtained incorporate all of the uncertainty in the prior of f and are unaffected by unreasonable estimates of f. Models were compared using the deviance information criterion (DIC), an analogue of Akaike's information criterion. Estimates of genetic diversity within each population (h_S hereafter; defined as average panmictic heterozygosity) were also obtained from Bayesian analyses. The statistical significance of differences between θ^B estimates for different population groups, or obtained from different models applied to the same data set, were tested by comparing the respective posterior distributions for the parameter (Holsinger and Wallace, 2004).

Analysis of Molecular Variance (AMOVA) (Excoffier *et al*., 1992) was used to partition total regional genetic diversity into its among and within-population components. Analyses were run for all populations, and separately for the lower, middle and upper altitude groups. Analyses were based on pairwise, individual-by-individual genetic distance matrices obtained using a simple-matching coefficient, in which any comparison with the same state yields a value of zero while any comparison of different states yields a value of unity. Although this coefficient may be inadequate in case of frequent band absence homoplasy, it was chosen here because its Euclidean metric properties make it particularly well-suited for its use in AMOVA (Bonin *et al*., 2007). Pairwise genetic distances among populations (Φ_{ST}, an analogue of F_{ST}; Excoffier *et al*., 1992) were also computed from the AMOVA, and their statistical significance was tested with permutation tests. The relative importance of gene flow and drift in determining genetic structure, as well as departures from equilibrium, were tested by plotting population pairwise Φ_{ST} values against geographical distances (Hutchison and Templeton, 1999; see also Slatkin, 1993; Rousset, 1997). Untransformed Φ_{ST} and distance values were used in the analyses to allow comparisons with Hutchison and Templeton's (1999) theoretical scenarios. Statistical significance of the Φ_{ST}–distance relationships was assessed using Mantel tests. Genetic differentiation among groups of populations at different altitudes was tested by running one further AMOVA with altitudinal groups and populations as hierarchically nested classification levels. AMOVA computations were done with GenAlEx version 6 (Peakall and Smouse, 2006).

Nonparametric regressions were used to assess the shape of the relationships of population size and within-population genetic diversity with altitude. In nonparametric regression, no assumptions are made regarding the specific form of the function linking the dependent and independent variable, other than that it is smooth. Cubic splines were fitted to the data that minimized a generalized cross-validation score, as described by Schluter (1988) and implemented in software GLMS version 4 (available at http://www.zoology.ubc.ca/~schluter/splines.html). Confidence intervals of fitted regressions were obtained by bootstrapping. Population size was found to vary with altitude, hence it was necessary to elucidate whether the observed altitudinal pattern in within-population genetic diversity actually depended on variation in altitude itself or was an indirect consequence of concomitant variation in population size. A generalized additive model (Hastie and Tibshirani, 1990) was fitted to the data using generalized cross-validation and SAS Procedure GAM (SAS Institute, 2004), with gene diversity as the dependent variable, and population altitude and size as two nonparametric smoothing terms. This made possible examining the shape of the relationship between genetic diversity and altitude while simultaneously accounting statistically for the possible influence of variations in population size.

Results

Size and frequency of populations

A total of 75 *L. latifolia* populations were found in the area surveyed in Sierra de Cazorla and Sierra del Pozo. The vast majority of populations were very small. Forty populations (53.3%) had <50 flowering individuals, 18 (24.0%) had between 50 and 250 and 17 (22.7%) had >250 (Figure 2). Only three (4%) large populations with >1000 individuals were found.

Populations occurred at altitudes between 1015 and 1605 m asl, being most frequent in the interval 1200–1400 m asl (Figure 2). The mean and spread of population size varied also with altitude. Average population size increased from the lower distributional limit to reach a peak at about 1300 m, and then declined as the altitude increased further. Populations around both the lower and upper altitudinal limits were invariably small. At middle altitudes, in contrast, the size of populations was quite variable and the whole range of population sizes were found. The few populations with >1000 plants were all found at intermediate altitudes (Figure 2).

Genetic structuring and diversity at the species' border

Populations differ widely in genetic diversity, as estimated by the proportion of polymorphic AFLP loci (%PL; range = 48.3–73.9%) and within-population gene diversity (h_S range = 0.141–0.199) (Table 1). Neither %PL ($r_s = 0.346$, $N = 21$, $P = 0.12$) nor h_S ($r_s = 0.199$, $N = 21$,

$P = 0.39$) is significantly correlated with population size in our sample. Estimates of genetic diversity do not vary clinally at the spatial scale of this study, as shown by nonsignificant rank correlations with geographical latitude ($r_s = 0.165$ and -0.008 for %PL and h_S, respectively; $N = 21$, $P \geqslant 0.47$) and longitude ($r_s = 0.203$ and -0.177 for %PL and h_S, respectively; $N = 21$, $P \geqslant 0.37$).

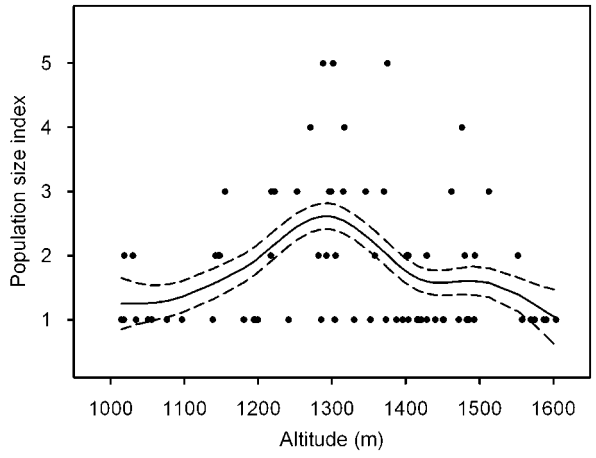

Figure 2 Altitudinal variation in the size of *Lavandula latifolia* populations in the surveyed area of Sierra de Cazorla and Sierra del Pozo. The nonparametric regression fitted to the data ($N = 75$ populations) is shown as a continuous line ± 1 s.e. of prediction (dashed lines). Population size index:1, <50; 2, 50–250; 3, 250–500; 4, 500–1000; 5, >1000 plants.

The Bayesian analysis on all populations revealed significant genetic differentiation. Examination of the DIC values of the models tested indicates that the full model should be preferred over the models with either $f = 0$ or $\theta^B = 0$ (Table 2). In the preferred full model, the posterior mean of θ^B was 0.298, with a fairly narrow 95% credible interval (95% CI hereafter) of 0.284–0.313. The estimate of f obtained from this model was 0.982 (95% CI = 0.933–0.999), which denotes nearly complete inbreeding. This result is biologically unreasonable, given that *L. latifolia* progenies are predominantly outcrossed (CM Herrera and P Bazaga, unpublished data), and presumably exemplifies another case of unreliable f estimation when Bayesian methods are applied to dominant marker data (Holsinger and Lewis, 2003). To examine whether the poor f estimate of the full model biased the θ^B value obtained, we also tested the free model, in which θ^B should be unaffected because the model did not involve the estimation of f. The θ^B value obtained in the free model (0.284) was also significantly different from zero (95% CI = 0.261–0.304), and was closely similar to the estimate provided by the full model, from which it did not differ significantly (95% CI of the difference of posteriors = −0.042 to −0.010) (Table 2).

The AMOVA-based estimate of population differentiation, $\Phi_{ST} = 0.305$, departed only slightly from the θ^B values obtained in the full and free models of Bayesian analyses, and was also statistically significant ($P < 0.001$; permutation test with 1000 repetitions). Population pairwise Φ_{ST} values were all significantly different from zero ($N = 210$ pairs, $P \leqslant 0.002$ in all cases; permutation

Table 1 Location and characteristics of the 21 *Lavandula latifolia* populations sampled

Population	*Longitude (°W)*	*Latitude (°N)*	*Altitude (m)*	*Population size*	N	*%PL*	h_S *(95% credible interval)*
Low altitude							
Garganta de Hornos	2.68874	38.20756	990	2	15	63.3	0.158 (0.147–0.169)
Ermita de Huéscar	2.58627	37.84042	1030	2	15	72.5	0.174 (0.163–0.188)
Raso del Tejar	2.87732	37.97938	1040	1	15	48.3	0.155 (0.145–0.166)
Los Villares	3.80820	37.65857	1080	1	15	67.6	0.155 (0.142–0.173)
Dehesa del Oso	2.58020	38.46459	1100	1	15	72.9	0.159 (0.148–0.174)
Las Navillas	2.91568	37.93299	1130	2	15	61.8	0.141 (0.130–0.154)
Puerto de Tíscar	3.02963	37.79218	1140	2	14	68.1	0.145 (0.134–0.157)
Middle altitude							
Cuevas Bermejas	2.85153	37.96558	1185	3	11	67.1	0.169 (0.158–0.182)
Presilla de Tíscar	3.00107	37.78327	1190	2	15	60.9	0.187 (0.177–0.198)
Aguaderillos	2.88578	37.96397	1210	3	15	73.9	0.168 (0.155–0.184)
Arroyo de los Ubios	2.90256	37.94004	1235	2	15	72.0	0.186 (0.172–0.203)
Peñascosa	2.33248	38.65407	1244	3	14	71.5	0.155 (0.142–0.173)
Tío Serafín	2.88353	37.96296	1280	5	12	70.5	0.199 (0.187–0.211)
Cruz de Quique	2.95391	37.90228	1290	2	14	62.8	0.185 (0.175–0.195)
High altitude							
Arroyo Amarillo	2.94000	37.88528	1380	2	15	65.7	0.181 (0.170–0.192)
Los Centenares	2.73838	38.06811	1391	2	15	71.5	0.171 (0.158–0.188)
Collado del Calvario	2.88548	37.95160	1425	1	15	56.0	0.172 (0.162–0.182)
Caballo de Acero	2.84212	37.90195	1450	2	15	65.2	0.147 (0.135–0.163)
Baza	2.84380	37.41050	1480	2	15	61.8	0.141 (0.130–0.152)
Los Escalones	2.58708	38.14614	1520	2	15	60.9	0.147 (0.137–0.157)
Navahondona	2.95767	37.85497	1540	3	15	66.7	0.165 (0.153–0.179)

Abbreviation: AFLP, amplified fragment length polymorphism.
Populations are listed in increasing order of altitude, and broken down into the three altitudinal groups recognized.
Population size classes 1–5 refer to the categories described in Materials and methods. *N*: number of plants used in AFLP analysis; %PL: percent polymorphic loci (out of a total of 207 polymorphic loci scored); h_S: within-population gene diversity, as estimated with the free model in the Bayesian analysis of all populations.

Table 2 Comparison of the four models tested in the Bayesian analysis of population genetic structure for the 21 populations of *Lavandula latifolia* studied

Model	f	θ^B	*DIC*
Full model	0.982 (0.933–0.999)	0.298[a] (0.284–0.313)	10353.11
$f=0$	—	0.235[b] (0.222–0.248)	10563.96
$\theta^B=0$	0.941 (0.770–0.998)	—	22187.50
Free model	—	0.284[a] (0.261–0.304)	10563.14

Abbreviation: DIC, deviance information criterion.
Values shown are the posterior means of f (an estimate of F_{IS}) and θ^B (an estimate of F_{ST}), along with their 95% credible intervals (in parentheses), and DIC (an inverse measure of the model's fit to the data). Estimates of θ^B sharing the same superscript do not differ significantly (differences tested by comparing posteriors, as described in the text).

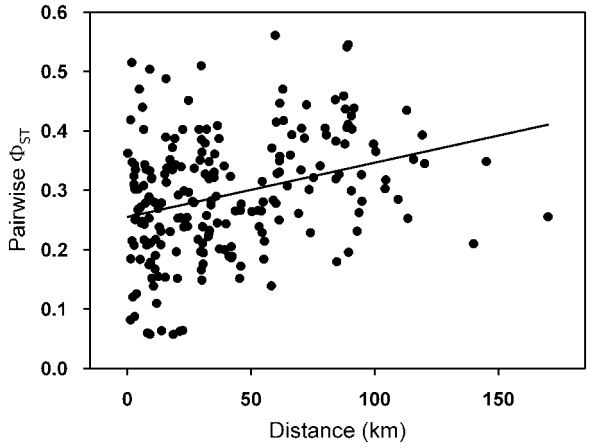

Figure 3 Relationship between pairwise Φ_{ST} (an AMOVA-based measure of genetic differentiation comparable to F_{ST}) and geographical distance for the 21 populations of *Lavandula latifolia* studied. The line shown is the ordinary least-squares regression ($y=0.255+0.0009x$).

tests with 1000 repetitions), and spanned one order of magnitude, ranging between 0.057 and 0.561. These results indicate that, although every population was genetically different from all the others, the magnitude of pairwise genetic distances was extraordinarily variable given the relatively small spatial scale of our study. Population pairwise Φ_{ST} increased with increasing geographical distance (Figure 3), and the relationship was statistically significant ($r=0.310$, $P=0.018$; Mantel test with 1000 permutations). There was, however, considerable scatter of points around the Φ_{ST} vs distance regression line, and variation in geographical distance explained only a minor fraction ($R^2=0.096$) of observed variance in pairwise Φ_{ST} values. Considerable variability of Φ_{ST} values took place even at the smallest geographical distances (Figure 3). For example, Φ_{ST} for the two nearest populations, which were only 0.25 km apart, was 0.362. For population pairs <4 km away, Φ_{ST} ranged between 0.081 and 0.515, a range comparable to that exhibited by all population pairs, some of which were >100 km apart.

Altitudinal pattern of genetic structuring and diversity

Within-population genetic diversity varies predictably with altitude, being significantly higher in middle than

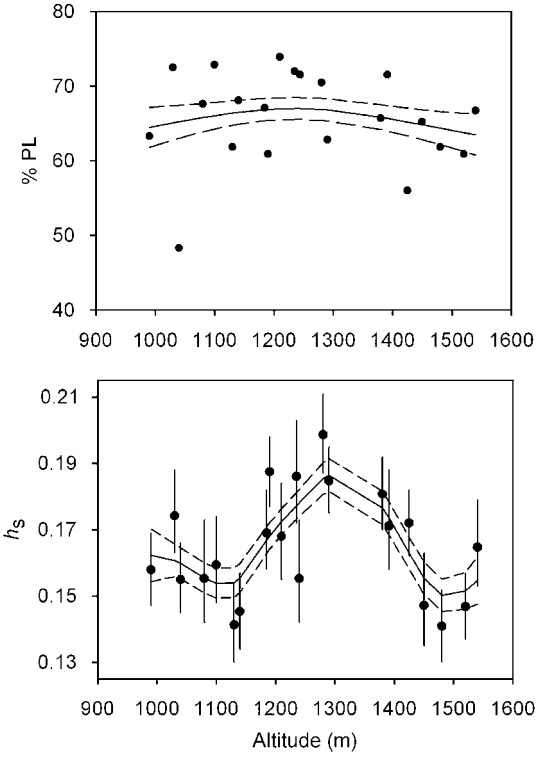

Figure 4 Variation with altitude of percent AFLP loci ($N=207$) that were polymorphic (%PL) and within-population gene diversity (h_S), two measurements of within-population genetic diversity, in the 21 *Lavandula latifolia* populations studied. In each graph, fitted nonparametric regressions are depicted as continuous lines, and dashed lines represent ± 1 s.e. of prediction. In the lower graph, vertical segments denote the 95% credible intervals of h_S estimates. AFLP, amplified fragment length polymorphism.

in upper or lower altitude populations (Table 1). This trend is particularly pronounced for variation in h_S, as shown by the nonoverlapping confidence intervals of the nonparametric regression at middle elevations in comparison to either upper or lower elevations (Figure 4). The trend revealed by the regression of h_S estimates against altitude is corroborated by the independent evidence provided by the 95% credible intervals around h_S. Credible intervals for most middle altitude populations are greater than, and largely nonoverlapping with, those for most upper and lower altitude populations (Figure 4). The pattern is also confirmed by the results of the generalized additive model simultaneously fitting the relationship between h_S (dependent variable) and altitude and population size as independent smoothing terms. After accounting for the nonsignificant linear ($t=0.18$, $P=0.86$) and marginally significant nonparametric ($\chi^2=3.5$, d.f. $=1.3$, $P=0.09$) effects of population size, the nonparametric component for the effect of elevation remained highly significant ($\chi^2=25.4$, d.f. $=6.4$, $P=0.0004$). After statistically removing the effects of variation in population size, the shape of the relationship between h_S and altitude was nearly identical to that shown in Figure 4 (graph not shown).

The extent of genetic differentiation among populations depends also on altitude. Separate Bayesian analyses conducted on the low-, middle- and high-altitude population groups reveal that the θ^B estimate for low-altitude populations (0.355) was significantly greater

Table 3 Genetic differentiation (θ^B and Φ_{ST}) and relationship between pairwise genetic and geographical distance at the three altitudinal groups of populations recognized in this study

Population group	θ^B	Φ_{ST}	Φ_{ST}—*distance regression*	
			R^2	P-*value*
Low	0.355[a] (0.321–0.388)	0.360	0.001	0.56
Middle	0.276[b] (0.243–0.308)	0.236	0.268	0.039
High	0.255[b] (0.223–0.286)	0.266	0.012	0.38

$N=7$ populations in each group. θ^B estimates were obtained by running separate free models for each population group. The 95% credible interval is shown in parentheses. θ^B values sharing the same superscript do not differ significantly, as tested by a comparison of posteriors. The AMOVA-based, Φ_{ST} values for the three population groups are all statistically significant ($P<0.001$; permutation tests with 1000 repetitions). Mantel tests with 1000 permutations were used to test the significance of the regressions of Φ_{ST} values against geographical distance.

than the corresponding values for middle- and high-altitude populations (0.276 and 0.255, respectively) (Table 3). A similar altitudinal pattern was revealed by Φ_{ST}, which was considerably greater for low-altitude populations than for either middle- or high-altitude ones (Table 3).

In addition to differing in genetic diversity and extent of population differentiation, the three altitudinal groups of populations also differ in the relationship between population pairwise Φ_{ST} and geographical distance. This relationship is statistically significant and accounts for a substantial proportion of variance in pairwise Φ_{ST} only for middle-altitude populations (Table 3). In contrast, the relationship is statistically nonsignificant and accounts for virtually no variance in pairwise Φ_{ST} in the low- and high-altitude populations (Table 3). Separate plots of Φ_{ST} against geographical distance for the low-, middle-, and high-altitude populations (not shown) reveal additional differences. High- and low-altitude populations, although similarly lacking a relationship between genetic and geographical distance, differ in the variance of pairwise Φ_{ST} values, which is considerably greater in the low- than in the high-altitude populations (0.0154 and 0.0048, respectively). It may thus be concluded that heterogeneity among altitudinal groups of populations in the shape of the relationship between pairwise genetic and geographical distance, and in the variance of pairwise Φ_{ST} values, acting in concert, is largely responsible for the considerable scatter of points when the relationship is evaluated for all populations combined (Figure 3).

There is no evidence of genetic differentiation among groups of populations at different altitudes, as shown by the AMOVA with altitudinal groups and populations as nested classification levels. The Φ values corresponding to differences among altitudinal groups and populations within groups are 0 ($P=0.60$) and 0.305 ($P<0.001$), respectively.

Discussion

Size and frequency of populations

Results of the survey of *L. latifolia* populations at the species' distribution edge are consistent with those expected from an ACD pattern, since populations are sparsely distributed and most of them consist of only a few tens of individuals. Furthermore, as implicated in ACD models (Hengeveld and Haeck, 1982; Brown, 1984), decreased abundance at peripheral locations is probably a consequence of suboptimal environmental conditions. In mediterranean climate regions of the Iberian Peninsula, summer seedling mortality caused by water stress is the main limitation to population recruitment in woody plants, including *L. latifolia* (Herrera, 2000; Rey and Alcántara, 2000), and this effect is particularly important in southern peripheral populations (Castro *et al.*, 2004; Arrieta and Suárez, 2006). *L. latifolia* conforms to this pattern, since summer seedling mortality is higher in the southern peripheral populations studied here (Herrera, 2000, 2002) than in a site close to the distribution center (Lloret *et al.*, 1999). In our study area, *L. latifolia* plants produce abundant viable seeds and suitable habitats are not rare, but the prevalence of small sparse populations is probably due to chronic limitations on seedling recruitment caused by severe water stress during the warm, dry summers characteristic of the southern Iberian Peninsula. This interpretation is supported by field experiments referred to in the next paragraph.

Variation in the number and size of *L. latifolia* populations with altitude is also consistent with the ADC model. Most populations occur within a relatively narrow belt at middle altitude, and the size and frequency of populations decline steadily from the altitudinal distribution center towards the upper and lower distribution edges, where very few, invariably small populations are found. The broad variation in population size occurring at middle elevations probably reflect heterogeneity in the size and/or age of suitable habitat patches, which would set upper limits to population size and age, respectively, but quantitative data are not available to support this possibility. Experimental studies conducted in the study region by Herrera (2000, 2002) suggest that altitudinal variation in ecological factors limiting seedling recruitment probably accounts for altitudinal variation in mean abundance. When *L. latifolia* seeds were sown experimentally in the field at sites distributed along an altitudinal gradient broader than, and encompassing the altitudinal range of the species, juvenile plants became established only at locations within the altitudinal range of the species. Altitudinally variable limitations on seedling establishment are thus most likely responsible for the altitudinal distribution of *L. latifolia* at its southern distribution limit.

Genetic structuring at the species' border

There was considerable genetic differentiation among populations, as revealed by the Bayesian and the AMOVA analyses. The two estimates of differentiation provided very similar values ($\theta^B=0.284$, $\Phi_{ST}=0.305$), revealing that among-population differences accounted for nearly one-third of total regional genetic diversity. The Φ_{ST} value obtained is very similar to the average of 0.35 reported by Nybom (2004) (Table 1) for 21 AFLP-based plant studies. Genetic differentiation of *L. latifolia* populations studied here, however, is probably greater than average, given the smaller spatial scale of this study in comparison to those in Nybom's review. Across studies, Φ_{ST} is strongly correlated with the maximum distance between sampled populations (Nybom, 2004),

and the mean maximum distance between sampled populations included in Nybom's compilation (1500 km) is one order of magnitude greater than the maximum distance in our study (170 km).

The small size of most *L. latifolia* populations, along with the short distances flown by insect pollinators and the lack of special seed dispersal mechanisms (Herrera, 1987), should combine to restrict gene flow among populations and make random genetic drift the most significant force in determining genetic structure at the species' southern range limit. In addition to reducing gene diversity within small populations, random genetic drift will also increase the between-population component of diversity (Ellstrand and Elam, 1993). This expectation is clearly supported by (i) the extensive genetic differentiation of populations at the reduced spatial scale of our study, as denoted by the high θ^B and Φ_{ST} values; (ii) the finding that every population was genetically different from each other ($\Phi_{ST} > 0$ in all pairwise comparisons); and (iii) the large genetic divergence between some populations ($\Phi_{ST} > 0.5$) despite the small geographical distances involved (Figure 3).

The characteristics of the scatterplot of pairwise genetic and geographical distances between populations strongly support our interpretation that random genetic drift is the prevailing force shaping the genetic structure of *L. latifolia* at its southern range edge. Although pairwise Φ_{ST} was significantly related to geographical distance, the slope of the regression was very shallow, and there was considerable scatter of points around the regression over the whole range of geographical distances, with variation in geographical distance explaining only 9.6% of variance in pairwise Φ_{ST} values. Assuming a stepping-stone model of population structure, Hutchison and Templeton (1999)(see also Slatkin, 1993) developed a model allowing to test for the existence of a regional equilibrium between drift and gene flow, and to elucidate the relative influence of drift and gene flow on population structure, by considering the characteristics of the scatterplot of genetic and geographical distances between populations. The scatterplot for the 21 *L. latifolia* populations studied here is closely similar to Hutchison and Templeton's Case III, which would correspond to a situation of lack of regional equilibrium where drift is much more influential on genetic structure than gene flow. In this scenario, allele frequencies in each population drift independently without any relation to the geographical distances separating them, and random sampling of gametes creates the wide degree of scatter between points. It must be noted, however, that no predictable relationship between genetic and geographical distances should be expected under a purely drift-driven, genuine Hutchison and Templeton's Case III scenario, yet a weak relationship does exist in our sample of *L. latifolia* populations. This seemingly anomaly is attributable to the effects of altitudinal gradients in population size and abundance, as discussed below.

Altitudinal patterns of genetic structuring

Previous researches on the genetic structure of peripheral plant populations have most often involved comparisons with populations at central locations of the area of distribution (Lammi *et al*., 1999; Lönn and Prentice, 2002; Eckstein *et al*., 2006). The present investigation has focused on peripheral populations alone. Although this approach precludes comparisons with central populations, our study encompasses a full altitudinal distribution, going from ecological periphery to core to periphery with elevation. This provides an analogy of a latitudinal gradient while controlling for factors such as regional climate, season length and colonization history. Furthermore, our sampling approach allows for spatially denser sampling and a finer-scale analysis of genetic structuring at the distribution edge. This was essential to our objective of dissecting the possible altitudinal effects on genetic structuring at the distribution border. As noted in Introduction, earlier studies considering the variation of genetic characteristics of plant populations along altitudinal gradients have provided heterogeneous results (Neale and Adams, 1985; Schuster *et al*., 1989; Premoli, 2003; Reisch *et al*., 2005). These investigations generally examined only a few populations unevenly distributed over, or incompletely encompassing, the altitudinal range of focal species. Apart from the perils of obtaining spurious results because of insufficient replication, sampling limitations may have reduced the statistical power of these studies to detect fine-scale altitudinal patterns of genetic structure and diversity, which might partly account for their inconsistent results. In this study on *L. latifolia*, a dense sampling evenly spaced over the whole altitudinal range of the species has revealed that both population-level genetic diversity (as measured by h_S) and degree of genetic structuring (as measured by θ^B and Φ_{ST}) vary along the species' altitudinal range. This study has also shown that, in mountainous landscapes, altitudinal genetic patterns may be an essential element to interpret the genetic structure of populations at species borders, as discussed below.

In *L. latifolia*, within-population genetic diversity varies predictably with altitude, being highest in middle-altitude populations and declining towards the upper- and lower-altitudinal margins. The extent of genetic differentiation among populations also vary with altitude. Although results differ slightly for θ^B and Φ_{ST}, genetic differentiation tends to increase from the central parts of the altitudinal distribution towards the upper and, particularly, the lower altitudinal belts. Altitudinal trends in genetic diversity and structure are most likely attributable to concomitant variation in the relative importance of genetic drift and gene flow due to changing size and density of populations. Genetic drift leading to erosion of local genetic diversity and enhanced population differentiation is expected to prevail at or near the upper and lower altitudinal margins because of the smaller size and sparser distribution of populations, randomness associated with founding events, and infrequent seed and pollen flow among populations. In contrast, a situation closer to a drift-gene flow equilibrium should be expected at the center of the altitudinal distribution, where populations are most abundant, considerably larger and gene exchange events via pollen or seeds should be less infrequent. This interpretation is strongly supported by the contrasting relationships of pairwise genetic vs geographic distance for populations at low, middle and high altitude. The high- and low-altitude population groups were similar in lacking a relationship

between genetic and geographic distances (R^2 of regressions = 0.001 and 0.012), thus unambiguously exemplifying two Case III scenarios in Hutchison and Templeton (1999) model. Populations at middle altitudes, in contrast, exhibited a statistically significant, positive relationship between genetic and geographic distance suggestive of a distinct pattern of isolation by distance, where limitations to gene flow contribute proportionally more than genetic drift to genetic differentiation (Hutchison and Templeton, 1999). These results highlight the most significant finding of this study, that is, altitudinal variation in the relative influences of gene flow and drift is an essential element to the interpretation of regional genetic structuring at *L. latifolia*'s distribution edge. The weak pattern of isolation by distance arising when all populations are analyzed together is misleading, since when the three altitudinal groups are analyzed separately, isolation by distance occurs only at middle altitude populations, while predominantly drift-driven patterns occur at both high and low altitudes. As shown also by Slatkin (1993) and Hutchison and Templeton (1999), the existence of population subsets that differ in the relative importance of drift and gene flow may, if unrecognized, lead to misleading interpretations of regional patterns of genetic structuring.

In addition to variation in the importance of genetic drift relative to gene flow, other factors may contribute to shape the relationship of genetic diversity and structure with altitude. At the regional level, *L. latifolia* populations most likely behave as a metapopulation subject to the dynamics of colonization and extinction, and metapopulation dynamics may also have contributed to shape patterns of genetic variation. Quantitative data on the age distribution of populations is not available, but anecdotal field observations conducted over two decades in Sierra del Pozo show that local populations may vary dramatically in size over 5–10 years, and that colonization and extinction events are not infrequent (CM Herrera, unpublished data). As found for metapopulations of other species (Barrett and Husband, 1997), temporal fluctuations in the size of local *L. latifolia* populations may account for the lack of relationship between genetic diversity and population size found in this study. Metapopulation dynamics may affect profoundly the spatial structuring and temporal dynamics of genetic variation (Pannell and Charlesworth, 2000; Whitlock, 2004), and some results of this study may be interpreted accordingly. As noted in Material and methods, populations on or near the upper and lower altitudinal limits seem to have shorter average lifespans and higher turnover rates than those at the center of the altitudinal range. If this possibility is substantiated by future studies, then the trend of increasing genetic differentiation from the center towards the edges of the altitudinal range would clearly conform with predictions from metapopulation models that incorporate variation in population age (McCauley *et al*., 1995). This scenario would correspond to a source-sink metapopulation system, with large, long-lasting, and genetically interconnected populations at the center of the altitudinal distribution behaving as sources, and small, short-lived, peripheral populations at lower and upper limits behaving as sinks. This interpretation is compatible with our finding of lack of genetic differentiation among groups of populations at different altitudes, as shown by the results of AMOVA with altitudinal groups and populations as nested classification levels.

Concluding remarks

The considerable diversity and high proportion of endemic taxa that characterize the flora of the Mediterranean region have been traditionally interpreted as reflecting enhanced speciation arising from recurrent episodes of geographical isolation and allopatric speciation promoted by the complex topography and geological history that characterize the area (Thompson, 1999; Sainz Ollero and Moreno Saiz, 2002). It has been recently suggested, however, that local speciation (*sensu* Levin, 1993), involving random genetic drift and the fixation of new, favorable gene combinations in novel ecological scenarios, may also have contributed significantly to the high plant species richness of the Mediterranean Basin (Thompson, 1999, 2005). Results of the present investigation are germane to this hypothesis. Major plant biodiversity hot-spots of the Mediterranean Basin, including the extraordinarily species-rich southern Spanish Betic Ranges where this study was conducted, are invariably associated with extensive and complex mountain systems (Médail and Quézel, 1997). In the Iberian Peninsula, plant diversity and endemism levels are greatest in mountainous regions spanning broad altitudinal ranges (Lobo *et al*., 2001; Rey Benayas and Scheiner, 2002). Local speciation is expected to be particularly important in metapopulations and/or isolated peripheral populations at the edges of species ranges (Levin, 1995). This study has shown that when species edges intersect mountainous regions, altitudinal ecological gradients may enhance population subdivision, hinder the establishment of drift-gene flow equilibria, and promote predominantly drift-driven patterns of genetic differentiation, all of which may favor local speciation. It may thus be speculated that, in a region characterized by so many large and complex mountain systems as the Mediterranean Basin, altitudinal ecological gradients may provide an additional mechanism favoring plant diversification through their contribution to population subdivision and genetic structuring at species edges.

Acknowledgements

We are grateful to E Cano, A Valido, and P Vargas for discussion and advice with AFLP laboratory protocols; C Fernández López for information on *L. latifolia* distribution; K Holsinger for advice on Hickory; C Alonso, M Medrano and two anonymous reviewers for useful comments; and Consejería de Medio Ambiente and Junta de Andalucía for authorizing this work and providing invaluable facilities in the Sierra de Cazorla region. Support for this work was provided by an agreement CSIC-Consejería de Medio Ambiente, and Grants RNM-156 and CGL2006-01355 from the Consejería de Innovación, Ciencia y Empresa, and Ministerio de Educación y Ciencia, respectively.

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