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ARTICLE

Phylogeography of two closely related species of *Nolana* from the coastal Atacama Desert of Chile: post-glacial population expansions in response to climate fluctuations

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ABSTRACT

Aim To investigate the impact of Quaternary climate cycles on the coastal Atacama Desert flora by assessing phylogeographical patterns of the desert shrub *Nolana crassulifolia* (Solanaceae) and its congener *Nolana incana*.

Location The latitudinal aridity gradient from the southern margin of the coastal Atacama Desert to the mediterranean semi-arid region of Chile (25–33° S).

Methods Two cpDNA regions were sequenced for 130 individuals in 15 populations of these two closely related species, covering their entire distribution range (1000 km) along the arid to semi-arid Chilean coast. We explored haplotype relationships in a statistical parsimony network, and assessed population genetic diversity, population differentiation and phylogeographical structure. In addition, we conducted demographic analyses and spatial analysis of genetic variation, identified barriers to gene flow with Monmonier's algorithm, and used Bayesian phylogenetic reconstruction to estimate divergence dates between lineages.

Results We found a total of 14 haplotypes – four of them shared by both species – and high levels of genetic differentiation among populations, but no past distribution breaks that could account for major vicariant events. Genetic diversity decreased continuously from north to south, with loss of haplotypes and a greater number of monomorphic populations in the southern range. Landscape analysis revealed greater genetic differentiation in the northernmost populations of both species.

Main conclusions The documented north–south gradient of declining genetic diversity, the origin and location of ancestral haplotypes in northern sites, and the loss of haplotypes from southern populations all support the hypothesis of post-glacial range expansion of *Nolana* southwards during arid/warmer cycles in the Atacama. The higher genetic diversity and greater differentiation of northern populations of both *Nolana* species support the hypothesis that populations survived in northern arid sites during wetter/colder episodes of the glacial cycles. We suggest that Quaternary arid phases in the Atacama promoted southward expansion of the coastal desert vegetation.

Keywords

Coastal Atacama Desert, cpDNA, *Nolana*, perennial shrub, plant phylogeography, post-glacial expansion, Quaternary, semi-arid region.

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INTRODUCTION

The Pleistocene was characterized by extensive perturbations of the global climate that strongly affected the distributions

of organisms and communities (Hewitt, 2000; Jackson & Overpeck, 2000; Williams *et al.*, 2004). Its effects on biogeographical patterns have been well documented for temperate, alpine and high-latitude regions (Taberlet *et al.*, 1998). At

arid subtropical latitudes, however, where the impact of glacial cycles was less evident, the results are not so conclusive or well documented (Lessa *et al.*, 2003). For the desert biome, the phylogeographical patterns associated with Quaternary climate cycles have been poorly explored, and research has focused primarily on animals (Riddle *et al.*, 2000; Jezkova *et al.*, 2009). The limited genetic evidence suggests that the distribution ranges of drought-resistant desert plants were strongly affected by glacial climate changes in the Northern Hemisphere (Nason *et al.*, 2002; Garrick *et al.*, 2009; Rebernig *et al.*, 2010) often supporting post-glacial range expansions. In contrast, we lack information on the glacial dynamics of distribution ranges for plants from the hyper-arid Atacama Desert of western South America, where intermittent episodes of high aridity interrupted by intervals of increased rainfall have been reported in the late glacial period (Latorre *et al.*, 2005).

The Atacama Desert of northern Chile, considered one of the driest places on Earth, extends along the western margin of South America from 17° to 30° S. The hyper-arid condition prevailing over much of the region dates back to the late Miocene (Dunai *et al.*, 2005), coeval with the final phase of central Andean uplift and intensification of the cold Humboldt Current (Placzek *et al.*, 2009). Coastal fog and winter rains caused by westerly storm fronts, modulated by the South Pacific Anticyclone (SPA), supply moisture to the southern margin of the Atacama, whereas summer rains transported by easterly winds are the principal source of rainfall in the Andean highlands. The Quaternary history of the Atacama Desert remains unresolved, although researchers agree that several wet episodes affected the Atacama during the last glacial–interglacial cycle (Betancourt *et al.*, 2000; Latorre *et al.*, 2005; Díaz *et al.*, 2012). At the time of the Last Glacial Maximum (LGM), easterly winds became weaker and westerly winds were displaced towards the equator (Lamy *et al.*, 1999), thus reducing summer rainfall in the northern Atacama, but intensifying winter rains at the southern margin of the desert (Maldonado *et al.*, 2005; Maldonado & Villagrán, 2006; Kaiser *et al.*, 2008). Moreover, a hypothetical scenario of weaker SPA during the LGM (Rojas *et al.*, 2009) probably led to a reduction of coastal fog in the Atacama, and the decline in the global ice accumulation in polar regions may have exposed a greater extent of the seafloor, thus creating new habitats for the coastal desert flora.

The coastal Atacama Desert in Chile exhibits an outstanding floristic diversity for a region of extreme aridity, with nearly 550 vascular plant species, 40% of them endemic to the region (Dillon & Hoffmann, 1997). Plant diversity in the hyper-arid core of the Atacama is largely restricted to fog-dependent ecosystems known as *lomas* formations, found on coastal mountain-tops above 600 m (Rundel *et al.*, 1991). At lower elevations, fog-free plant communities are also present, and are maintained by sporadic seasonal precipitation, which becomes more regular south of 26° S along the transition from arid to semi-arid (Armesto *et al.*, 1993).

Range contraction and isolation in multiple refugia during wet/cold episodes during glacial–interglacial cycles might have played a major role in promoting population divergence and vicariant speciation in the Atacama Desert flora. However, genetic evidence of population fragmentation is only available for *Dioscorea biloba* (Viruel *et al.*, 2012), a desert plant that currently has a disjunct distribution close to the margins of the Atacama Desert. Very little is known about the response to Pleistocene climatic oscillations of desert plants with currently continuous distributions across the southern margin of the Atacama. It can be postulated that these plant species should have been less affected by climate changes during glacial–interglacial cycles, or alternatively, that they could have tracked semi-arid conditions, contracting their ranges northwards during wet/cold episodes and expanding southwards during warmer and drier periods.

One of the most representative and diverse plant genera of the coastal Atacama Desert is *Nolana* (Solanaceae), with about 85 species and subspecies of annuals, perennial herbs and shrubs distributed in the western margin of the southern Peruvian and northern Chilean deserts, extending southwards into the semi-arid mediterranean region of Chile (Dillon, 2005). The shrub *Nolana crassulifolia* Poepp. is among the most widespread species in the genus, extending across the arid to semi-arid transition from 27° to 33° S, along the Pacific shoreline. Individuals of this species closely resemble the related *Nolana incana* (Phil.) Johnston. (Johnston, 1936), which occupies the same habitats; the latter species is smaller in size and grows at the northern end of the range (25°–27° S), in hyper-arid conditions. Recent studies of phylogenetic relationships within the genus *Nolana*, using four chloroplast DNA regions (Tu *et al.*, 2008) and the nuclear regions *GBSSI* (Dillon *et al.*, 2007) and *LEAFY* (Tu *et al.*, 2008) have not completely resolved the relationship between *N. crassulifolia* and *N. incana*. However, the combination of molecular and morphological data suggests that *N. crassulifolia* and *N. incana* are sister species, constituting a monophyletic clade (see Materials and Methods and Appendices S1–S2 in Supporting Information). The crown age established for the entire genus *Nolana*, 4.02 Ma, dates back to the Pliocene, followed by diversification in the early Pleistocene, from 2.88 to 1.25 Ma (Dillon *et al.*, 2009). Thus, the history at population level of these two closely related species with a broad distribution in the southern margin of the Atacama Desert might provide evidence for plant responses to glacial–interglacial climatic fluctuations in the Atacama.

In the present study, we used DNA sequence data from two chloroplast DNA (cpDNA) regions to infer the population responses of these two closely related *Nolana* species to glacial–post-glacial climatic fluctuations in the southern Atacama Desert. Based on the palaeoclimatic scenario described above for the southern Atacama Desert, we expected to find evidence for contraction of the species' ranges to northern latitudes during wetter/colder episodes of the last glacial cycle, and document a post-glacial population expansion to occupy the southern part of the range, concurrent with the

increased aridity during the Holocene. Recent population expansions are predicted to cause a north-to-south trend of decreasing genetic diversity for both closely related species, with large areas of the present distribution fixed for a single haplotype near the southern margin of the geographical range. This genetic pattern could result from founder effects and successive population bottlenecks (Hewitt, 1996). In addition, we tested for evidence of population fragmentation and isolation that should be reflected in geographically structured haplotypes, with high levels of genetic differentiation between populations and isolation by distance. We treated all the populations of the two closely related species, *N. incana* and *N. crassulifolia*, distributed at the southern margin of the Atacama and in the Chilean semi-arid region, as components of a hypothetical single evolutionary unit that responded similarly to climate fluctuations.

MATERIALS AND METHODS

Species, study sites and plant collections

The study was focused on *N. crassulifolia* and *N. incana*. Both species inhabit the rocky sea shores of the Pacific coastal desert, are prostrate subshrubs with succulent leaves and a small white funneliform (bell-shaped) corolla (Fig. 1). Their seeds are located in 5–9 small rounded mericarps that remain inside the fruit until germination. Mericarp dispersal is basically passive, mostly persisting in the accrescent calyx, but some mericarps may be dispersed by wind or water (ocean). Although no reports of animal dispersal are known, fossil mericarps of *Nolana* spp. have been collected from ancient rodent middens (Betancourt *et al.*, 2000; Díaz *et al.*, 2012).

The phylogenetic relationship between the two species is still unresolved (Dillon *et al.*, 2007; Tu *et al.*, 2008). Chloroplast data placed both in an unresolved clade that also includes other Chilean endemic shrub species (clade FH in Tu *et al.*, 2008). Nuclear data (Dillon *et al.*, 2007; Tu *et al.*, 2008) placed *N. crassulifolia* and *N. incana* in the same clade as *N. divaricata*, *N. peruviana* and *N. werdermannii*. In order to clarify the phylogenetic relationships within the clade including the two species, we reconstructed a total-evidence tree using 11 morphological traits (Appendix S1) and concatenated DNA sequences for the Chilean shrub species of *Nolana* (clade FH in Tu *et al.*, 2008). We only included individuals with data for the six DNA markers (named according to collector's vouchers). For morphological data, we examined three individuals per species from samples deposited at the Museo Nacional de Historia Natural in Santiago, Chile (MNHN; herbarium code SGO), and descriptions in the literature (Johnston, 1936; Dillon *et al.*, 2007). Results supported with 91% confidence (bootstrap value) the close sister relationship between *N. crassulifolia* and *N. incana* (Appendix S2).

The sampled range in our study extended along the littoral zone of the mediterranean region, covering 1000 km between

25° S and 33° S, and including the complete geographical range of the focal taxa. Plant material was collected from 14 localities, belonging to three populations of *N. incana*, 10 of *N. crassulifolia*, and one sympatric site. Sample size ranged from two to 22 individuals (130 plants in total), according to population density; in every locality, we defined transects of 200 m along the coast from where samples were taken. All populations from which samples were taken were georeferenced and relevant vouchers from localities were deposited at MNHN.

DNA extraction and sequencing of non-coding regions of cpDNA

Dried leaf tissue was ground in a TissueLyser (Qiagen, Valencia, CA, USA); total genomic DNA was extracted using DNeasy Plant Mini Kit (Qiagen) and verified on 2% agarose gels. We tested nine individuals for amplification and polymorphism in 12 non-coding cpDNA regions. The intergenic spacers *rpl32-trnL* and *rpl16* intron showed the highest variability and were amplified for the full sample. Amplifications were carried out following Shaw *et al.* (2005, 2007), using 12.5 µL GoTaq Colorless Master Mix (Promega, Madison, WI, USA), 0.5 µM each primer, 20 ng of DNA template, and nuclease-free water (Promega) to reach a final volume of 25 µL. PCR products were verified on 1% agarose gels and sent to Macrogen (Seoul, South Korea) for purification and sequencing with forward and reverse primers. Difficult PCR products were purified using QIA-quick purification kit (Qiagen) and sequenced in the Molecular Diversity Laboratory of Pontificia Universidad Católica (Chile). Sequences were aligned and edited with CLC SEQUENCE VIEWER 6.6 (CLC Bio A/S, 2008); indels due to poly-N were ignored. All sequences were deposited in GenBank, with accession numbers KC434177–KC434307 (*rpl32-trnL*) and KC434308–KC434438 (*rpl16*).

Data analysis

Genetic diversity

The number of haplotypes (*K*), haplotype (gene) diversity (*H_d*), nucleotide diversity (π) and the average number of pairwise nucleotide differences per site (*II*; Nei, 1987) were estimated using DNASP 5.1 (Librado & Rozas, 2009). To assess possible founder effects and find evidence of the latitudinal migration southwards from a past distribution further north, we analysed the trends of within-population genetic diversity with latitude. We subdivided the distribution range into three approximately equal geographical regions, based on mean climatic conditions (di Castri & Hajek, 1976; Luebert & Plischoff, 2006): North (25–27° S; mostly hyper-arid; mean annual precipitation: < 25 mm yr⁻¹), Central (27–30° S; mostly arid; mean annual precipitation: 29–108 mm yr⁻¹) and South (30–33° S, semi-arid; mean annual precipitation: 120–350 mm yr⁻¹). To account for

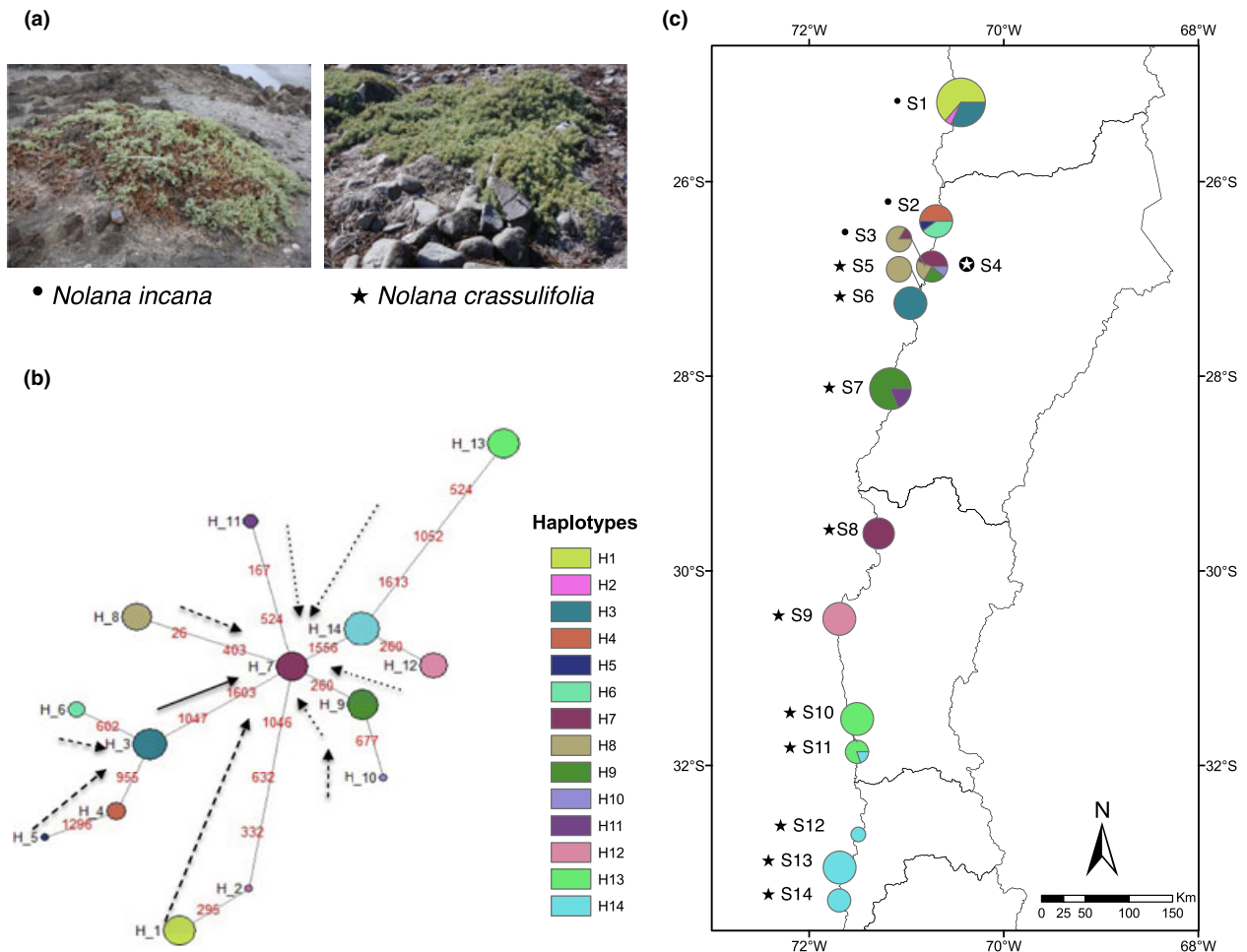


Figure 1 Geographical distribution and genealogical relationships of the 14 cpDNA haplotypes recovered from 14 populations of *Nolana incana* and *N. crassulifolia* in the Atacama Desert, Chile. (a) Habitus of *N. incana* and *N. crassulifolia*. (b) Statistical parsimony network; circle sizes and branch lengths are proportional to haplotype frequencies and the number of mutational steps between haplotypes, respectively. Dashed, dotted and solid arrows represent the three different contraction events according to the star-contraction algorithm. (c) Geographical map of the sampling locations, with pie charts displaying the frequency of occurrence of each haplotype in each locality; pie chart size is proportional to population size, and haplotype colours correspond to those shown in panel (b). The symbols beside localities denote populations of *N. incana* (black circle), *N. crassulifolia* (star) or both (star inside a circle).

differences in sample size among populations, we performed Monte Carlo simulations to standardize population sizes to $n = 9$ and recalculated the genetic diversity estimators mentioned above. These procedures were performed with an R script and the R package PEGAS 0.4-1 (Paradis, 2010).

Network representation

Relatedness among haplotypes was represented by a statistical maximum parsimony network (MP) using the software tcs (Clement *et al.*, 2000), and a median-joining (MJ) network with the software NETWORK 4.6.1.0 (Bandelt *et al.*, 1999). This software was also used to run the star contraction algorithm that simplifies networks estimating historical demographic expansion events (Forster *et al.*, 2001). A minimum spanning tree (MST) was calculated using Prim's algorithm (Prim, 1957) with HAPSTAR (Teacher & Griffiths, 2011).

Populations differentiation and geographical structure analyses

Genetic differentiation among populations was evaluated using the G_{ST} and N_{ST} coefficients (Pons & Petit, 1996). Both coefficients estimate the ratio between the mean within-population genetic diversity and total genetic diversity, but while the G_{ST} index makes use only of the allelic frequencies, N_{ST} also takes into account the genetic distances among haplotypes. These indices were statistically compared using 1000 permutations in PERMUTCPSSR 2.0 (Pons & Petit, 1996). A greater N_{ST} means that more closely related haplotypes occur in the same population, indicating phylogeographical structure. Only populations with a sample size larger than three individuals were considered. A spatial autocorrelation analysis (SAA) and a Mantel test were performed with GENALEX 6.1 (Peakall & Smouse, 2006) to identify

patterns of isolation by distance. SAA was run with distance intervals of 82 km, equal to the average distance between sampling locations, and a 95% confidence interval around the null hypothesis of no spatial structure was calculated using 1000 permutations. Affinities between the populations were assessed considering genetic and geographical distances using spatial analysis of molecular variance (SAMOVA) with SAMOVA 1.0 (Dupanloup *et al.*, 2002). Populations with fewer than two individuals were excluded from the analysis. To test for neutral evolution, we calculated the mismatch distribution, Tajima's *D* and Fu's *F_S* using ARLEQUIN 3.5.1.3 (Excoffier & Lischer, 2010). The significance of these tests was evaluated as the proportion of random statistics less than or equal to the observed value. For positive values of Tajima's test, we used the published critical values (Tajima, 1989).

A genetic landscape shape analysis was performed using ALLELES IN SPACE (Miller, 2005). This analysis identifies genetic discontinuities among populations in a landscape shape and produces a three-dimensional surface plot where the *x* and *y*-axes correspond to the geographical coordinates of populations, and the *z*-axis corresponds to the interpolated genetic distances. We investigated the genetic barriers associated with each geographical location and populations, using Monmonier's maximum-difference algorithm, implemented in BARRIER 2.2 (Manni *et al.*, 2004). Significance of barriers was estimated using 10,000 bootstrapped pairwise distance matrices generated in R (R Development Core Team, 2012).

Bayesian divergence time estimations

To estimate divergence times between haplotypes, we performed a Bayesian analysis using BEAST 1.7.4 (Drummond *et al.*, 2012) with a log-normal relaxed clock, extended Bayesian skyline tree prior and TVM+I substitution model, which was selected using the Akaike information criterion (AIC) as implemented in jMODELTEST 2.1.1 (Darriba *et al.*, 2012). Given the lack of fossil records for *Nolana*, we calibrated the clock using a strong prior on the substitution rate (Drummond *et al.*, 2006). Using the available data published in a previous independent study (Dillon *et al.*, 2009), we estimated a mean of 2.23×10^{-9} substitutions per site per year ($s\ s^{-1}\ yr^{-1}$) and a standard deviation (SD) of $0.6 \times 10^{-9}\ s\ s^{-1}\ yr^{-1}$; this value lies within the mutation rate interval described for most angiosperms ($1.0\text{--}3.0 \times 10^{-9}\ s\ s^{-1}\ yr^{-1}$, see Wolfe *et al.*, 1987). We ran three independent MCMC analyses for 40,000,000 generations sampling every 4000. Logs and tree files were combined in LOGCOMBINER 1.7.4 (Drummond *et al.*, 2012).

We verified the convergence of the estimated parameters and that the effective sample sizes were at least 200 in TRACER 1.5 (Rambaut & Drummond, 2007); trees were summarized in TREEANOTATOR 1.7.4 (Drummond *et al.*, 2012) with 10% burn-in, and edited in FIGTREE 1.4 (Rambaut, 2012).

RESULTS

Haplotypes and network analysis

The alignments of the *rpl32-trnL* and *rpl16* data sets of *Nolana* were 720 and 929 bp in length, respectively, including two variable mononucleotide repeats. The 1649-bp combined data set contained 18 nucleotide substitutions. A total of 14 haplotypes were identified among the 130 individuals sampled from 10 populations of *N. crassulifolia* and three populations of *N. incana* at the southern margin of the Atacama Desert. The two species occurred in sympatry at only one site. Four haplotypes (H3, H7–H9) were shared between populations of the two closely related *Nolana* species.

Six of the 14 haplotypes we identified occurred in more than two *Nolana* populations, with frequencies of 10–14% (Fig. 1). Three haplotypes were very rare, with frequencies below 1%. The most widely distributed haplotypes in the entire sample were H14, H7 and H3. Haplotype H14 was present in four *Nolana* populations (S11–S14) within a range of 170 km and was restricted to the South (semi-arid) region; haplotypes H7 and H3 were found in three (S3, S4 and S8) and two (S1 and S6) populations, respectively, occurring in both the North and Central portions of the species' range and encompassing a geographical range of 300 km and 218 km, respectively.

Network constructions of haplotype structure with the MJ and MP algorithms showed the same results (Fig. 1). The network presented a loop involving four haplotypes (H7, H14, H12, H9), which was broken between H12 and H9 in both trees. The central and most connected haplotype was the widespread haplotype H7, which was inferred to be the most likely ancestral haplotype. A maximum of four mutational steps separated H7 from the remaining haplotypes, with the longest branches formed by haplotypes H13–H14 (from the southernmost populations) and haplotypes H1–H2 (from the northernmost populations). The star contraction algorithm suggested stepped demographic expansion events that converged in H7. The first contraction shrank haplotypes H6, H4 and H5 into H3; H1, H2 and H8 into H7; and H10 into H9. The second contraction shrank all remaining haplotypes into H7, except for H3.

Population differentiation and phylogeographical structure

The coefficient of differentiation N_{ST} (0.81) was high, and significantly higher than G_{ST} (0.76) (permutation test: $P = 0.04$), indicating significant population differentiation and suggesting the existence of phylogeographical structure. The Mantel test ($r = 0.38$; $P = 0.001$) and the spatial autocorrelation analysis (SAA) ($\omega = 165.786$; $P = 0.001$) were both significant, thus supporting the hypotheses of isolation by distance. The correlogram showed a general trend of decreasing autocorrelation (r) with increasing geographical distance (Appendix S3); despite this, a smaller increment in r at intermediate distances (about 300 km) was detected,

which is probably a consequence of low genetic differentiation among populations in the southern portion of the distribution range.

Based on the cpDNA haplotypes and matrix of geographical distances, SAMOVA identified five phylogeographical groups with an $F_{CT} = 0.63$ (Fig. 2). These five groups, however, did not represent clearly separated geographical units. The northern populations (S1–S5) were disaggregated into four groups: Group 1, containing only the northernmost site (S1); Group 2, including both S2 and the central S6; Group 3, connecting S3 and S5; and Group 4, connecting S4 with all central and populations located south of 28° S, except S10 and S11, which together comprise the final Group 5. Monmonier's algorithm also revealed strong differentiation among northern populations. The three major genetic barriers were located in the north between 25° and 27° S (Fig. 2), with bootstrap values that sum to 92.4%. These barriers isolate S1 from S2 (26.4%), S2 from S3–S5 (58.2%), and S6

from the northernmost populations (7.8%). Genetic landscape analysis was congruent with these results and showed an overall reduction in genetic distance among *Nolana* populations from north to south, delimiting two zones of pronounced differentiation (Fig. 3). The first was found at the extreme north and was composed of three peaks (25.8°, 26.7° and 27.1° S), and the second was smaller and located at the southern margin of the range, at around 32° S.

The Bayesian chronogram of haplotypes showed a geographical pattern of divergence closely congruent with SAMOVA groupings (Fig. 2). The ages of divergence of haplotype lineages were estimated at between 0.7 and 0.15 Ma in all cases, corresponding to the mid-Pleistocene period.

Latitudinal patterns of genetic diversity

Genetic diversity varied markedly among the populations (Table 1), but was higher in the northernmost cases. The

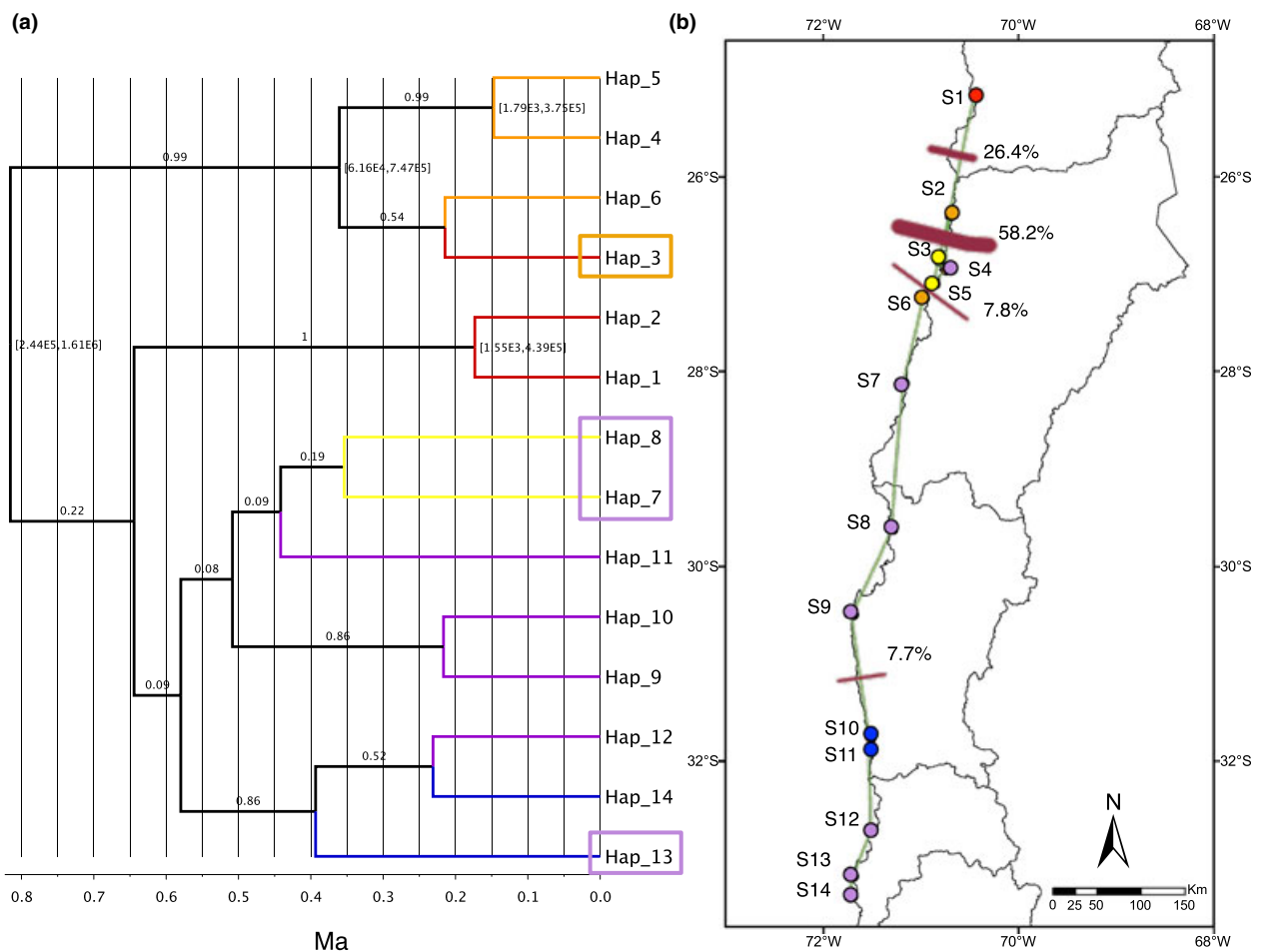


Figure 2 Geographical relationships among haplotypes of *Nolana* in Chile. (a) Bayesian chronogram of haplotypes; axis scale represents million years ago (Ma); numbers above branches represent posterior probabilities; only 95% intervals of nodes with posterior probabilities > 0.9 are shown; branch colours correspond to those shown in panel (b) and represent the SAMOVA groups in which these haplotypes were found; highlighted haplotypes are those shared between populations that belong to different groups. (b) Barriers obtained with Monmonier's algorithm and SAMOVA groups are shown on the map; the thickness of each barrier is proportional to the number of times it was included in one of the 10,000 computed barriers from bootstrap matrices and expressed as percentages; SAMOVA groups are represented by the colours red (Group 1), orange (Group 2), yellow (Group 3), purple (Group 4) and blue (Group 5).

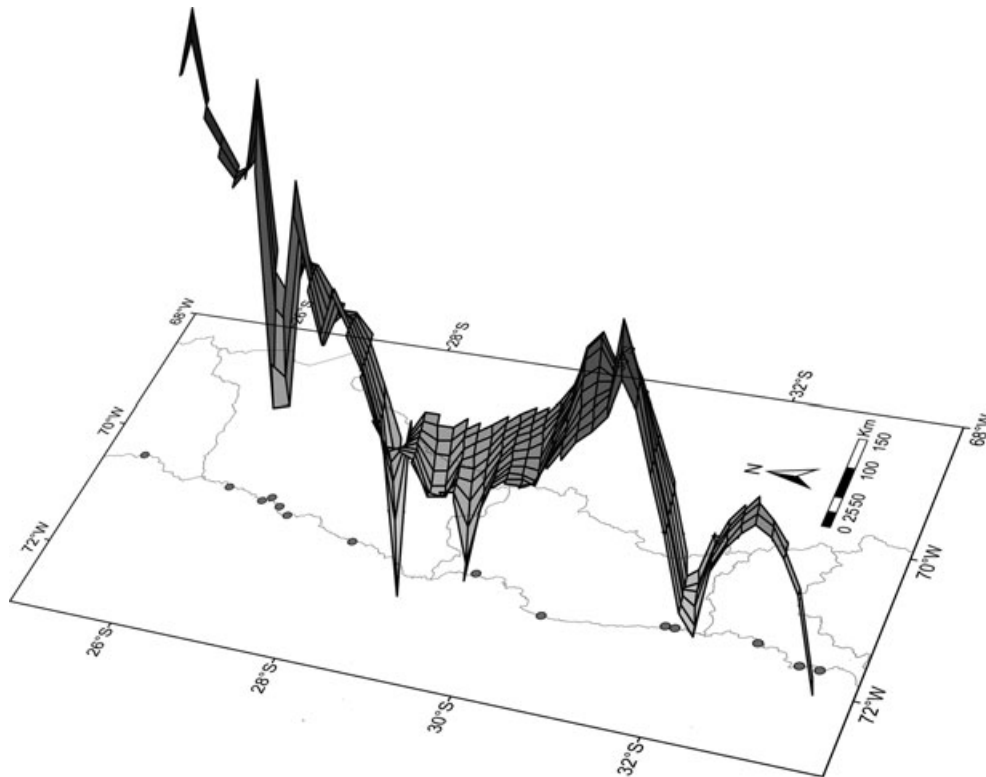


Figure 3 Genetic landscape analysis showing the latitudinal trend of differentiation between *Nolana* populations. The landscape is standardized with a grid of 50×30 cells comprising the entire geographical range of the close relatives *Nolana crassulifolia* and *N. incana* in all localities, superimposed on a map of the southern Atacama Desert. Peaks and depressions of the surface are proportional to genetic distances.

highest haplotypic diversity (Hd) was found in S2 and S6, situated at latitudes 26.4° and 26.9° S, whereas mean pairwise differences (Π) among individual plants were highest in the northernmost site situated at latitude 25.1° S. Eight populations were fixed for a single haplotype, and five of them occurred south of 30° S, in the semi-arid region. When differences in sample size among populations were considered (Fig. 4), excluding the smaller populations, and the genetic diversity index was standardized for $n = 9$, we detected a significant reduction of within-population genetic diversity with increasing latitude (Hd : $r = -0.73$, $P = 0.012$, $n = 9$; Π : $r = -0.70$, $P = 0.017$, $n = 9$). The same tendency was observed when populations were pooled into three a priori geographical groups: North (north to 27° S), Central (27° – 31° S) and South ($> 31^\circ$ S) (Table 2).

Demographic analyses

The pairwise mismatch distribution for the entire sample was unimodal, with a maximum at 4 bp, and did not differ significantly from the null population expansion model (Table 3). However, the more conservative estimate of population expansion, Tajima's D , was non-significant, and therefore, did not indicate a departure from population equilibrium ($D = 0.111$, $P = \text{n.s.}$). When the analysis was

performed for the SAMOVA groupings, Groups 2–5 showed unimodal mismatch distributions, but only Group 5 showed a significant negative value for Tajima's D , suggesting population expansion (Group 2: $D = 0.11$, $P = \text{n.s.}$; Group 3: $D = -1.45$, $P = 0.08$; Group 4: $D = -0.26$, $P = \text{n.s.}$; Group 5: $D = -1.68$, $P = 0.01$). Group 1 – containing only the northernmost population S1 – had a bimodal mismatch distribution with peaks at 0 and 6 bp, as well as a significantly positive Tajima's D value ($D = 2.09$, $P < 0.05$), suggesting population contraction.

DISCUSSION

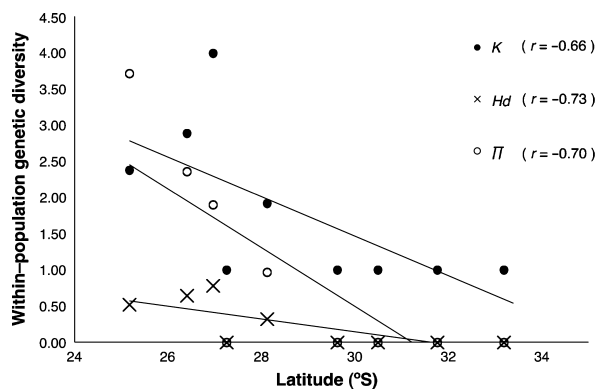
Chloroplast DNA markers did not recover the distinction between *N. crassulifolia* and *N. incana*. The finding of four haplotypes shared by both species supports the hypothesis of a recent divergence between these two closely related taxa and incomplete lineage sorting. Therefore, it seems legitimate to analyse the phylogeography of *N. crassulifolia* and *N. incana* together, treating them as a single evolutionary complex using cpDNA (see for example Grivet & Petit, 2002).

We detected high levels of genetic differentiation among *Nolana* populations, and significant phylogeographical structure (high $G_{ST} = 0.76$ and $N_{ST} > G_{ST}$), but SAMOVA did not produce an arrangement of sites in discrete geographical

Table 1 Genetic diversity of *Nolana* populations found at the southern margin of the Atacama Desert. Location, latitude, longitude, voucher number, climate, sample size (*n*) and measurements of genetic diversity for three populations of *N. incana*, 10 of *N. crassulifolia*, and one sympatric site (S4).

Code	Species	Locality	Climatic region	Lat. (°S)	Long. (°W)	Collector voucher	<i>n</i>	<i>K</i>	<i>Hd</i>	Π	π
S1	<i>N. incana</i>	Bandurrias	North	25.18	70.44	Ossa 267	22	3	0.51 (0.08)	2.76 (1.52)	0.0017 (0.001)
S2	<i>N. incana</i>	Playa Hippie	North	26.41	70.70	Ossa 251	10	3	0.64 (0.10)	1.27 (0.87)	0.0008 (0.0006)
S3	<i>N. incana</i>	Zenteno	North	26.87	70.81	Ossa 107	6	2	0.33 (0.21)	0.67 (0.59)	0.0004 (0.0004)
S4	Sympatric	Q. El León	North	26.96	70.74	Ossa 315, 321	9	4	0.78 (0.11)	1.50 (0.99)	0.0009 (0.0007)
S5	<i>N. crassulifolia</i>	Bahía Inglesa	Central	27.11	70.85	Ossa 114	6	1	0	0	0
S6	<i>N. crassulifolia</i>	Bahía Cisne	Central	27.25	70.96	Ossa 237	10	1	0	0	0
S7	<i>N. crassulifolia</i>	Hualtata	Central	28.13	71.17	Ossa 256	16	2	0.33 (0.13)	0.98 (0.70)	0.0006 (0.0005)
S8	<i>N. crassulifolia</i>	Hornos	Central	29.62	71.29	Ossa 171	9	1	0	0	0
S9	<i>N. crassulifolia</i>	Talcaruca	South	30.49	71.69	Ossa 201	10	1	0	0	0
S10	<i>N. crassulifolia</i>	Chigualoco	South	31.76	71.51	Ossa 157	10	1	0	0	0
S11	<i>N. crassulifolia</i>	Playa Amarilla	South	31.86	71.51	Ossa 286	5	2	0.40 (0.24)	1.20 (0.91)	0.0007 (0.0006)
S12	<i>N. crassulifolia</i>	Caucau	South	32.71	71.50	Ossa 302	2	1	0	0	0
S13	<i>N. crassulifolia</i>	Quintay	South	33.18	71.69	Ossa 210	10	1	0	0	0
S14	<i>N. crassulifolia</i>	El Quisco	South	33.39	71.69	Ossa 086	5	1	0	0	0
Total							130	14	0.90 (0.01)	3.44 (1.77)	0.0021 (0.0012)

K, number of haplotypes; *Hd*, haplotypic diversity; Π, mean pairwise differences; π, nucleotide diversity. Standard deviations are given in parentheses.

**Figure 4** Latitudinal variation of within-population genetic diversity in *Nolana* at the southern margin of the Atacama Desert and the transition to mediterranean climate. *K*, number of haplotypes; *Hd*, haplotypic diversity; Π, mean pairwise differences. Sample sizes were standardized to *n* = 9 for the populations shown.

units as expected under a hypothesis of biogeographical breaks related to vicariance. The present geographical distribution of the *Nolana* complex could be the result of several population contractions/expansions rather than fragmentation of an extended ancient population, as proposed in the only phylogeographical study available for an Atacama Desert plant (Viruel *et al.*, 2012). Both *Nolana* species are restricted to the rocky coastline, showing a nearly continuous distribution over 1000 km, and the current level of genetic differentiation among the populations studied is most probably the result of population isolation derived from habitat discontinuities along the coast. A geographical pattern similar to *Nolana* has been documented for other rock-dwelling shore-

line taxa, such as macroalgae (Fraser *et al.*, 2010), rock-pool copepods (Burton & Feldman, 1981) and also terrestrial plants (Cain *et al.*, 2000). These taxa often exhibit significant levels of genetic differentiation despite usually being able to disperse over long distances. Accordingly, a clear isolation-by-distance signal was found in SAA for short geographical distances (0–250 km), strongly biased by the high differentiation shown by the northern populations.

The conspicuous latitudinal gradient in both genetic differentiation and genetic diversity of *Nolana* – along the transition from hyper-arid to semi-arid – is also supported by genetic landscape analysis and barriers detected by Monmonier's algorithm, pointing to marked population differentiation at 25–27° S. Northern populations within this species complex also showed the highest number of haplotypes and greater haplotypic and nucleotide diversity, suggesting that they have been able to persist for long time. These data support the hypothesis that populations within this species complex remained in northern areas during the glaciations and wet Holocene episodes, when increased runoff or groundwater upwelling could have allowed the expansion of local populations of *Nolana* along creeks and mountain slopes, as suggested for lomas vegetation by Díaz *et al.* (2012). Exposed seashore as a consequence of lower sea level also favoured population expansion in lowland areas (Faure *et al.*, 2002; Sakaguchi *et al.*, 2010). In fact, Monmonier analyses grouped coastal sites S3, S5 and the inland S4 – located in the head of a dry stream bed – together, reflecting past and present interconnections by alluvial fans in coastal Atacama. During Holocene and interglacial arid episodes, populations in the hyper-arid Atacama probably contracted, as a consequence of an overall trend of declining rainfall and groundwater discharge, as well as rising sea

Table 2 Within-population mean and total genetic diversity for *Nolana* populations occurring in three climatic regions at the southern margin of the Atacama Desert.

Climatic regions	Mean or total	No. of individuals	No. of populations	<i>K</i>	<i>S</i>	<i>Hd</i>	II	π
North (25–27° S)	Mean within-population	27	3	3.1	4.33	0.65 (0.12)	2.65 (2.67)	0.0016 (0.0011)
	Total	47	4	10	13	0.85 (0.03)	3.82 (1.95)	0.0023 (0.0001)
Central (27–30° S)	Mean within-population	27	3	1.3	2	0.11 (0.05)	0.32 (0.40)	0.0002 (0.0002)
	Total	41	4	5	7	0.78 (0.03)	1.99 (1.15)	0.0012 (0.0001)
South (30–33° S)	Mean within-population	27	3	0	0	0	0	0
	Total	42	6	3	4	0.66 (0.002)	1.74 (1.03)	0.0011 (0.0001)

K, number of haplotypes; *S*, number of polymorphic sites; *Hd*, haplotypic diversity; II, mean pairwise differences; π , nucleotide diversity. Standard deviations are given in parentheses. For mean within-population diversity in each climatic region, sample sizes were standardized to $n = 9$.

Table 3 Patterns of demographic expansion for five groups of Chilean *Nolana* defined by SAMOVA. Standard deviations are given in parentheses. Mismatch distributions were evaluated under a model of demographic expansion and spatial expansion using the sum of square differences (SSD) between the observed and the expected mismatch as a test statistic.

	<i>n</i>	<i>K</i>	II	Tajima's <i>D</i>	<i>P</i> -value	Fu's <i>F_S</i>	<i>P</i> -value	Demographic expansion model		Spatial expansion model	
								SSD	<i>P</i> -value	SSD	<i>P</i> -value
Group 1	22	3	3.21 (1.72)	2.093	< 0.05†	5.473	0.978	0.450	0.000	0.129	0.062
Group 2	20	4	1.61 (1.00)	0.105	0.607	1.170	0.746	0.205	0.049*	0.179	0.013*
Group 3	12	2	0.50 (0.46)	−1.454	0.084	1.054	0.597	0.040	0.063	0.018	0.244
Group 4	61	7	1.42 (0.88)	−0.247	0.452	−0.569	0.402	0.008	0.101	0.008	0.069
Group 5	15	2	0.53 (0.47)	−1.685	0.013*	1.318	0.714	0.026	0.060	0.012	0.228
Total	130	14	3.44 (1.77)	0.111	0.595	−0.267	0.469	0.004	0.492	0.005	0.604

*Significant by coalescent simulations.

†Significant following published critical values (Tajima, 1989).

levels. Populations could have been restricted to rocky beaches and the foothills of coastal mountains where fog and marine spray can be trapped. Accordingly, Tajima's *D* is significantly high in the northernmost population S1, suggesting recent population contraction. Expansion/contraction cycles could have occurred several times over the Quaternary, producing remixing of lineages. Some lineages could have expanded southwards into the semi-arid region when aridity became widespread at the southern margin of the Atacama.

In contrast to populations from the extremely arid north, southern populations showed lower genetic variation, with most populations fixed for a single haplotype, as could be expected for colonizing populations. Reduced genetic diversity within southern populations of *Nolana* is consistent with the prediction derived from founder effects, as successive population bottlenecks during range expansion tend to reduce diversity (Hewitt, 1996). Furthermore, Group 5, composed of populations S10 and S11, revealed higher genetic diversity than their closest neighbours, with the presence of an ancestral and a derivative haplotype, suggesting long-term persistence of taxa during adverse periods in small habitat refugia followed by population mixing. This finding is supported by values of Tajima's *D* that suggest recent population expansion.

Long-distance dispersal into mediterranean climate areas can probably be assisted by seabirds (see Gillham, 1956), as plants of *Nolana* are commonly found close to seabird nesting sites (P.G.O., pers. obs.; see also Bernal *et al.*, 2006), or by oceanic dispersal, given the characteristics of plants and fruits (vegetative growth and mericarp buoyancy). One type of barrier for the continuous southward expansion of the distribution range of *Nolana* could be a river estuary interrupting terrestrial connectivity. The Copiapó River, the first major east–west river crossing the latitudinal gradient occupied by *Nolana*, could be a barrier for seed dispersal during humid periods. During arid phases of the glacial and post-glacial, dispersed seeds of *Nolana* could presumably cross these barriers, but river flooding during humid periods could promote isolation. Such barriers would explain the allele fixations that occur in the southernmost populations studied. In fact, the outlet of the Maipo River – one of the largest rivers in central Chile – represents the southern end of the current distribution range of *N. crassulifolia*.

The hypothesis of north-to-south migration raised here is also supported by the gradual divergence pattern observed in the Bayesian chronogram of haplotypes (Fig. 2). This result suggests that several migratory events occurred from north to south along the coast, followed by local northward

recolonization. The calculated divergence dates position the split of haplotypes before the last glacial period, in the mid-Pleistocene, indicating that the current distribution could be the product of climatic fluctuations in the last glacial period and the Holocene.

CONCLUSIONS

Overall, we provide genetically and phylogeographically based evidence that populations of two closely related species in a *Nolana* species complex persisted at northern locations of their geographical range during glacial events, accumulating much genetic diversity, which is still evident today. During drier periods associated with the interglacial phases, the northernmost populations became restricted to moist/foggy coastal refugia, while the southern range expanded following higher temperatures and greater aridity. This sequence of events may have recurred during glacial–interglacial cycles, promoting lineage mixing. Some populations remained isolated in southern habitats, showing signs of expansion today. These patterns are in agreement with the classic scenario of post-glacial range expansion documented for the North American desert floras (Nason *et al.*, 2002; Clark-Tapia & Molina-Freaner, 2003; Rebernig *et al.*, 2010), where wetter and colder glacial conditions triggered the contraction of drought-resistant desert plant species to lower, more arid latitudes. To our knowledge, this is the first study to offer genetic evidence of post-glacial range expansion of a desert plant in the southern Atacama Desert. Although no fossil records are available to document contraction/expansion events for *Nolana*, palaeoclimatic reconstructions for the last glacial–interglacial cycle (Maldonado & Villagrán, 2002, 2006; Kaiser *et al.*, 2008; Díaz *et al.*, 2012) tend to support our conclusions.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 States of morphological characters used in phylogenetic analyses of 12 Chilean shrub *Nolana* species.

Appendix S2 Majority-rule consensus tree of 12 Chilean shrub *Nolana* species based on Bayesian analysis of morphological characters and combined molecular data.

Appendix S3 Spatial correlogram illustrating the isolation-by-distance pattern.

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