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METHODS AND RESOURCES ARTICLE

# Population differentiation in relation to conservation: nuclear microsatellite variation in the Canary Island endemic *Lotus sessilifolius* (Fabaceae)

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**Abstract** We developed and characterized microsatellite markers for the genus *Lotus*, a large genus of leguminous plants containing many endemic species of conservation interest. The marker system was then used to survey patterns of population genetic variation of *Lotus sessilifolius*, a Canary Island endemic occurring on four islands (La Palma, El Hierro, La Gomera and Tenerife) with the aim of determining whether any of its populations are worthy of special conservation because of genetic distinctiveness. We found strong differentiation between populations with conspicuous geographical signal revealed by population clustering. Generally, populations from each island grouped together. A very striking exception to this pattern is a single population from Tenerife (Tejina-Milán: Anaga Peninsula), which is separated from other Tenerife populations by both genetic clustering and a STRUCTURE analysis, and also shows signs of inbreeding. The genetic distinctiveness of this population deserves especial conservation attention, and may be related to the ancient geological history of the Anaga Peninsula. Importantly, this investigation sets the stage for conservation genetics research in other highly threatened species in the same genus.

**Keywords** Phylogeography · Macaronesia · Simple sequence repeat · SSR

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## Introduction

Macaronesian *Lotus* L. (Fabaceae: Loteae) is a group of herbaceous legumes currently comprising some 40 species distributed in the 5 oceanic archipelagos of this region (Canary Islands, the Azores, the Cape Verde islands, Madeira and Selvagens), and of Morocco (Sandral et al. 2006). The group colonized Macaronesia from an ancestor of mainland Africa, and has radiated into a wide variety of habitats from sea level (lowland scrub) up to 2000 m (high Canarian mountains) (Allan et al. 2004).

Contrasting with many other congeneric Canarian endemics, *Lotus sessilifolius* is unusual in its widespread distribution, currently encompassing four islands in the Canary Island archipelago: Tenerife, La Palma, El Hierro and La Gomera (Sandral et al. 2006). This species is currently subdivided into two subspecies. *L. sessilifolius* DC. subsp. *villossisimus* (Pitard) Sandral and Sokoloff is exclusively distributed in El Hierro, while *L. sessilifolius* DC. subsp. *sessilifolius* is found in Tenerife, La Palma and La Gomera (see Fig. 1). In all the islands, this species is distributed in lowland scrub habitats from sea level to ca. 500 m. *L. sessilifolius* produces hermaphrodite autogamous yellow flowers adapted to insect pollination (*Bombus canariensis* Pérez). Fruits are commonly found in all the populations we investigated, and the plants grown under nursery conditions produced viable seeds even in the absence of pollinators. Furthermore, this taxon is of

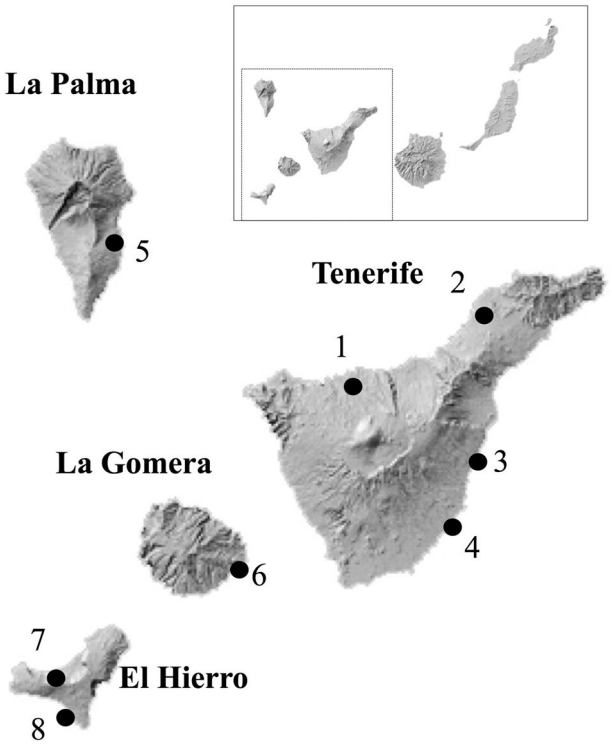


**Fig. 1** Map showing the distribution of *Lotus sessilifolius* in the Canary Island archipelago (*square*) with the four westernmost islands enlarged. *Numbers* indicate the approximate sites where each population of *L. sessilifolius* was collected. *Lotus sessilifolius* ssp. *sessilifolius*: 1 = Barranco de Ruiz (Tenerife), 2 = Tejina-Milán (Tenerife), 3 = Güimar (Tenerife), 4 = Poris de Abona (Tenerife), 5 = Mazo (La Palma), 6 = Puntallana (La Gomera); *L. sessilifolius* ssp. *villosissimus*: 7 = Punta de Arenas Blancas (El Hierro), 8 = Tecorone (El Hierro)

particular interest in that it has been shown to be a close relative of the apparently bird-pollinated *Lotus* species on the Canary Islands: the Rhyncholotus group (Ojeda et al. 2012).

Microsatellite markers have been widely used to study the genetics of plant populations (Ashley 2010; Kramer et al. 2011), and they have given very important information in a wide variety of Canarian endemic lineages (Sosa et al. 2010; García-Verdugo et al. 2015; Mairal et al. 2015). Our main objectives in this study are (1) to develop nuclear microsatellite markers (SSR) for this particular species and (2) to apply these SSR markers to investigate the intraspecific genetic variation in *L. sessilifolius*. Although it is a widespread taxon, it consists of many discrete populations fragmented by the separation between islands, and by topographic features within islands. Thus, we also wish to determine whether any of these populations are particularly distinctive at the genetic level and worthy of especial conservation status. These markers will also be useful for studies of other *Lotus* species in the Canary Islands.

## Materials and methods

### Study species and DNA isolation

We carried out comprehensive sampling of a key taxon of Macaronesian *Lotus: L. sessilifolius* DC., throughout its range of distribution. Leaf material was collected from all four islands in the Canary Island archipelago in which it occurs: La Palma, Tenerife, La Gomera and El Hierro (see Fig. 1) and preserved in silica gel. Six individuals per population and a total of eight populations were sampled. Voucher specimens are deposited in the herbarium of the University of British Columbia (UBC). The geographical location of these eight populations is shown in Table 1. Total genomic DNA was extracted using a modified version of the CTAB method (Doyle and Doyle 1987).

### Microsatellite selection and amplification

*Lotus sessilifolius* microsatellites were developed by cross-species amplification of primers designed from *Lotus japonicus* sequences (Sato et al. 2008), from which we selected 37 microsatellites. Further information about the sequences in relation to the whole genome sequence of *L. japonicus* is given in Sato et al. (2008). We designed

**Table 1** Localities of the eight populations of *Lotus sessilifolius*

| Subspecies | Population | Elevation (meters above sea level) | Coordinates |
|---|---|---|---|
| ssp. *sessilifolius* | Barranco de Ruiz, Tenerife | 5–10 | 28R 340943 E/3142073 N |
| ssp. *sessilifolius* | Polígono Industrial de Güimar, Tenerife | 40 | 28R 365537 E/3134772 N |
| ssp. *sessilifolius* | Poris de Abona, Tenerife | 15 | 28R 359821 E/3114448 N |
| ssp. *sessilifolius* | Tejina-Milán, Tenerife | 20–30 | 28R 366698 E/3158501 N |
| ssp. *sessilifolius* | Playa el Pocito, Mazo, La Palma | 20–30 | 28R 230423 E/3166005 N |
| ssp. *villosissimus* | Punta de Arenas Blancas, El Hierro | 10 | 27R 783621 E/3074679 N |
| ssp. *villosissimus* | Tecorone, El Hierro | 125 | 27R 793616 E/3064585 N |
| ssp. *sessilifolius* | Puntallana, La Gomera | 5–10 | 28R 293326 E/3113071 N |

primers in conserved flanking regions of these microsatellites using Primer3 software (Rozen and Skaletsky 2000). Of these, 11 amplified successfully in *L. sessilifolius*. PCR amplifications were performed in a final volume of 15 µl, containing 10 ng of DNA, 1× PCR buffer (10 mM Tris–HCl pH 8.3, 50 mM KCl), 2.5 mM of each dNTP, 1.5 mM $MgCl_2$, 1 U of Taq DNA polymerase, 0.05 µM forward primer, 0.5 µM reverse primer, and 0.5 µM dye-labeled universal primer. For all 11 loci, the forward primer was synthesized with the universal M13 sequence: TGTAAAACGACGGCCAGT. Four dyes were used VIC, FAM, NED and PET (Applied biosystems, Carlsbad CA, USA) for multiplexing. PCR reactions were performed using a BIO-RAD thermocycler with a touch-down program: 95 °C for 3 min, followed by 9 cycles of 94 °C for 30 s, 65 °C (decreased by 1 °C per cycle) for 30 s and 72 °C for 45 s, followed by 29 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s, and finally followed by a final extension at 72 °C for 20 min. Each microsatellite primer pair was amplified singly and then pooled together based on differences in fluorescent labeling and expected fragment size. An ABI 3730 automated DNA Sequencer (Applied Biosystems) was used to genotype the multiplex sets. The software GeneMapper v.3.2 (Applied Biosystems) was used to score the allele sizes.

### Microsatellite data analysis

Genetic variation within *L. sessilifolius* was determined using the 11 polymorphic microsatellite loci with a total of 48 individuals representing all main populations and taxonomic subdivisions. Due to *L. sessilifolius* being a tetraploid species, we used computer programs specifically designed for polyploidy analyses. Pairwise genetic distances between all samples were calculated using the R package software POLYSAT (Clark and Jasieniuk 2011). Two genetic distance measures were calculated: Lynch distance (Lynch 1990) and Bruvo distance (Bruvo et al. 2004). Lynch's distance is based on band-sharing dissimilarity between individuals, and Bruvo's distance takes into account mutations (Clark and Jasieniuk 2011). Both the Lynch's and Bruvo's genetic distance measures showed very similar genetic relationships among the 48 individuals. We used the Lynch's distance neighbor joining tree to show the individual-based intraspecific genetic relationships. The APE software (Paradis et al. 2004) was used to construct a neighbor joining tree of the 48 individuals based on Lynch distance.

We calculated population genetics statistics using the software AUTOTET (Thrall and Young 2000). For each of the eight populations we calculated allelic richness ($A$), observed ($H_O$) and expected heterozygosity ($H_E$) and inbreeding coefficient ($F_{IS}$). The values of $H_E$ and $F_{IS}$ were calculated based on random chromosomal segregation (Thrall and Young 2000).

Population allele frequencies were calculated using the 'simpleFreq' method in POLYSAT, which assumes all alleles have the same chance of being present in more than one copy (Clark and Jasieniuk 2011). Then these allele frequencies were used to estimate pairwise genetic distances ($F_{ST}$) among the eight populations of *L. sessilifolius*. The APE software (Paradis et al. 2004) was used to construct population-based trees using various algorithms (UPGMA and neighbour-joining) and various population distance metrics (Nei's $D$, $G_{ST}$, and $F_{ST}$). UPGMA and neighbour-joining (NJ) are complementary: UPGMA assumes clock-like behaviour of microsatellite variation whereas NJ does not, but UPGMA is very robust when evolutionary rates are high, as in microsatellites (Huelsenbeck and Kirkpatrick 1996). Various measures have been used to compute genetic distances between populations, such as the commonly used $F_{ST}$, Nei's standard genetic distance ($D$), and "unbiased genetic distance" ($G_{ST}$) (Nei 1972; Goldstein et al. 1995; Petren et al. 1999; Ochieng et al. 2007).

Population genetic structure was also calculated using the software STRUCTURE 2.3.4 (Pritchard et al. 2000). STRUCTURE is complementary to population clustering. It is not appropriate for inferring the genetic relatedness of distinct populations but can be used for the assignment of individuals to populations and for inferring the existence of distinct populations (Pritchard et al. 2000). We performed 20 replicate runs for all $K$ values using the admixture model and correlated allele frequencies among populations. We used a length of 500,000 burn-in period and 750,000 Markov chain Monte Carlo repetitions, and $K$ values ranging from 1 to 8. The best inference of K was determined using the method of Evanno et al. (Evanno et al. 2005) based on the maximized second-order rate of change in posterior probability for a given $K$ (i.e. $\Delta K$).

## Results

### Microsatellite variation

Overall, the microsatellite loci were highly polymorphic in the 48 individuals of *L. sessilifolius* sampled. The number of alleles per locus ranged from two alleles to 21 alleles with an average of 10.82 alleles per locus (Table 2). The patterns of microsatellite alleles per locus per individual were consistent with the tetraploid nature of *L. sessilifolius*, ranging from one to four.

**Table 2** Characteristics of all successfully amplified microsatellite primers in *Lotus sessilifolius*, obtained by cross species amplification from conserved regions of the SSR flanking regions of *Lotus japonicus* (Sato et al. 2008)

| Primers | Primer sequence (5′–3′) | Repeat | Allele size range (bp) | *Na* | Ta |
|---|---|---|---|---|---|
| TM1894 | F: GTGGATAGCGATATGGAGTTTGA<br>R: GGCGTGGTTCTTTTGACATT | $(AAG)_8$ | 266–287 | 10 | 55 |
| TM0512 | F: GGAAGTGAGCAAGGAAGAAGC<br>R: GTCGCAATTTCCTACACACA | $(AAG)_8$… $(AAG)_7$ | 260–287 | 12 | 55 |
| TM0219 | F: GTCATCACCATCCCTATCTTCTC<br>R: GCGGTGATCTTAACCTTGGA | $(TTCA)_3$… $(TTC)_{11}$ | 259–284 | 21 | 55 |
| TM0060 | F: ACGTGCAGTCCCTGTTAAGC<br>R: CCAATTGCCACATCCCTTAC | $(CT)_{24}$ | 300–328 | 15 | 55 |
| TM1170 | F: GGCGGTTTCTTGACACATTC<br>R: TTCCAGGTTTTCACCAGCAT | $(GT)_{23}$ | 220–244 | 2 | 55 |
| TM0831 | F: CAGCTCTGCCAAGATCAACA<br>R: CTGCTGCCTGAGCTGTGTAT | $(CA)_{20}$ $(CT)_{26}$ | 279–303 | 13 | 55 |
| TM0164 | F: CCCTCGAGTCCACTTCCAT<br>R: CCTAGCTTCATCCAGAATTGG | $(CT)_{14}$ | 195–221 | 12 | 55 |
| BM1881 | F: TGCAGTTGGTCTTCCGGTTT<br>R: CACCAGCATTCCATGTTTCA | $(CT)_{16}$ | 172–192 | 12 | 55 |
| TM0680 | F: GGCTAATTCCTTCACCGTCA<br>R: AAAGCCTGCCGTGGTATTC | $(ATT)_{18}$ | 206–240 | 4 | 55 |
| TM0456 | F: TACCACCTGAGCCATTGTTG<br>R: GGCGATTTCTCCAAACCTAC | $(AGG)_{14}$ | 174–223 | 14 | 55 |
| TM0233 | F: ACGTTGAGCCTCCTCAACTG<br>R: GAGCCAGTTATCCATGTTTG | $(AGG)_{24}$ | 224–228 | 4 | 55 |

For each primer, the total number of alleles (*Na*), and annealing temperature in °C (Ta) are shown together with other information

## Population genetic diversity

Population level genetic diversity estimates were similar across the eight populations except for the population Tejina-Milán in Tenerife (pop C), which had the lowest genetic diversity values (Table 3). This population had an allelic richness (A) of 1.09, observed heterozygosity of 0.05 and had the highest inbreeding coefficient ($F_{IS}$) estimate at 0.31, suggesting that it is highly inbred.

In contrast, the other seven populations had allelic richness ($A$) ranging from 2.11 to 2.52, observed heterozygosity ranging from 0.51 to 0.64, and inbreeding coefficient ($F_{IS}$) ranging from 0.04 to 0.13, indicating that they are predominantly outbred.

## Genetic differentiation

The genetic relationships among the 48 individuals showed a strong phylogeographical signal. Individuals from distinct

**Table 3** Population genetic diversity values for *Lotus sessilifolius*

| Pop ID | Populations | $A$ | $H_O$ | $H_E$ | $F_{IS}$ |
|---|---|---|---|---|---|
| A | Playa el Pocito, Mazo, La Palma | 2.11 ± 0.71 | 0.51 ± 0.28 | 0.58 ± 0.24 | 0.13 ± 0.1 |
| B | Punta de Arenas Blancas, El Hierro | 2.32 ± 0.61 | 0.59 ± 0.19 | 0.66 ± 0.20 | 0.11 ± 0.09 |
| C | Tejina-Milán, Tenerife | 1.09 ± 0.25 | 0.05 ± 0.13 | 0.07 ± 0.13 | 0.31 ± 0.19 |
| D | Puntallana, La Gomera | 2.20 ± 0.80 | 0.53 ± 0.30 | 0.57 ± 0.28 | 0.08 ± 0.10 |
| E | Poris de Abona, Tenerife | 2.52 ± 0.80 | 0.64 ± 0.26 | 0.68 ± 0.22 | 0.06 ± 0.08 |
| F | Polígono Industrial de Güimar, Tenerife | 2.46 ± 0.85 | 0.60 ± 0.30 | 0.65 ± 0.26 | 0.07 ± 0.12 |
| G | Tecorone, El Hierro | 2.24 ± 0.92 | 0.54 ± 0.33 | 0.56 ± 0.30 | 0.04 ± 0.16 |
| H | Barranco de Ruiz, Tenerife | 2.24 ± 0.72 | 0.57 ± 0.27 | 0.63 ± 0.27 | 0.09 ± 0.12 |

For each population the allelic richness ($A$), observed ($H_O$) and expected heterozygosity ($H_E$) and the inbreeding coefficient ($F_{IS}$) values are shown with their standard deviations. Population identifiers (ID) are the same as those given in Fig. 1

populations generally clustered together in the neighbor joining tree (Fig. 2). An exception was found in the individuals from Poris de Abona (pop E) and Güimar (pop F). Most of the individuals from these two populations grouped together, likely due to high gene flow between the two populations. Another interesting pattern is the grouping of two individuals from Poris de Abona (pop E) with two individuals from Punta de Arenas Blancas (pop B), possibly indicative of a gene exchange event in the past. The former is located in Tenerife and the latter in the island of El Hierro.

Pairwise genetic distance measures between the eight populations based on $F_{ST}$ and other metrics showed that populations from the same islands clustered together (Figs. 3, 4). In the UPGMA and neighbour joining trees the populations from each island generally group together, with the exception of population Tejina-Milán (C), which is located on the Anaga peninsula in Tenerife and is genetically distinct from all the other *L. sessilifolius* populations.

Population genetic structure was analyzed using the Structure software (Fig. 5). The best inference of $K$ (maximum $\Delta K$) occurred at $K=7$ with a value of 89.0, separating all the populations except the adjacent Tenerife populations E and F which appear as a single population. These two populations are located on the eastern mountainside of Tenerife (i.e. Güimar and Poris de Abona) and appear to have considerable gene flow between them. In comparison the next largest peak was $K=3$ with a value of 8.7. At $K=3$ the Tejina-Milán population (C) was grouped with the La Gomera population (D) and is thus

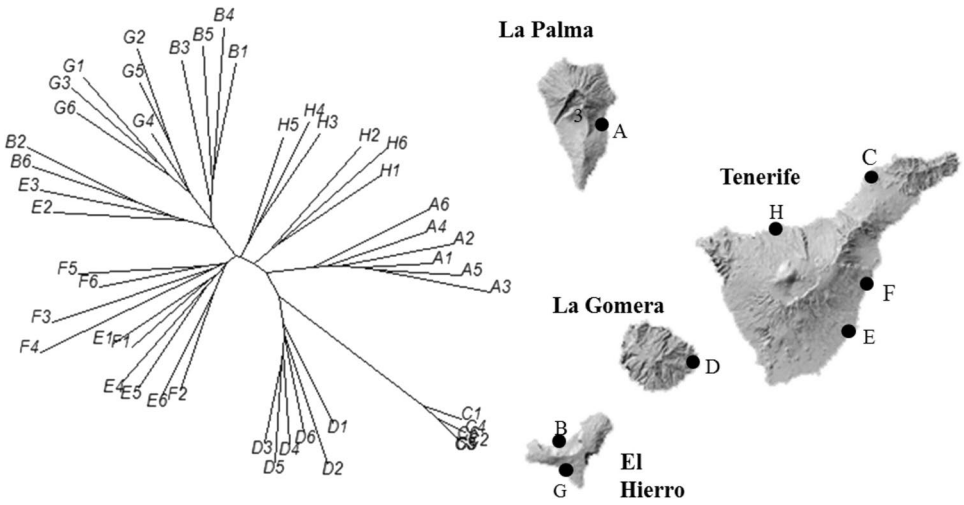


**Fig. 2** Neighbor joining (NJ) tree showing the relationships of all 48 *L. sessilifolius* samples based on the 11 microsatellite loci. The genetic distances were calculated using the Lynch distance method (Lynch 1990). *Key* A = Mazo (La Palma), B = Punta de Arenas Blancas (El Hierro), C = Tejina-Milán (Tenerife), D = Puntallana (La Gomera), E = Poris de Abona (Tenerife), F = Güimar (Tenerife), G = Tecorone (El Hierro), H = Barranco de Ruiz (Tenerife)

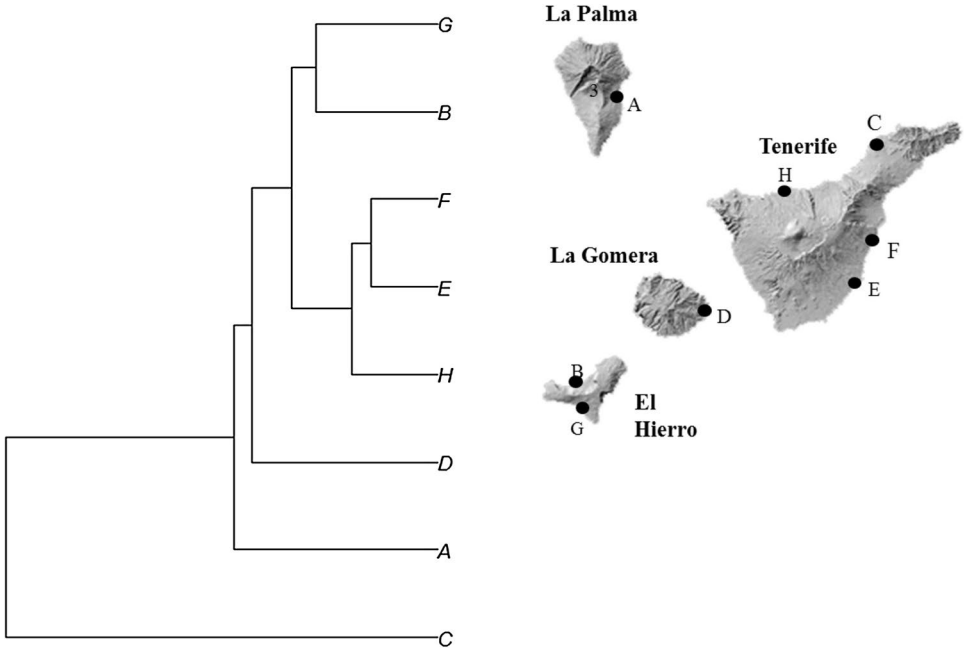


**Fig. 3** Dendrogram (UPGMA) based on $F_{ST}$ pairwise genetic distances calculated using the software POLYSAT. *Key* A = Mazo (La Palma), B = Punta de Arenas Blancas (El Hierro), C = Tejina-Milán (Tenerife), D = Puntallana (La Gomera), E = Poris de Abona (Tenerife), F = Güimar (Tenerife), G = Tecorone (El Hierro), H = Barranco de Ruiz (Tenerife)

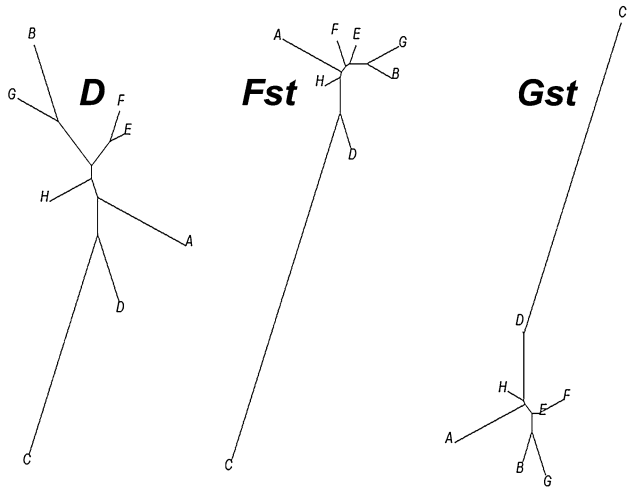


**Fig. 4** Unrooted neighbour-joining trees showing the genetic relationships of the populations based on various distance measures. *Key* A = Mazo (La Palma), B = Punta de Arenas Blancas (El Hierro), C = Tejina-Milán (Tenerife), D = Puntallana (La Gomera), E = Poris de Abona (Tenerife), F = Güimar (Tenerife), G = Tecorone (El Hierro), H = Barranco de Ruiz (Tenerife)

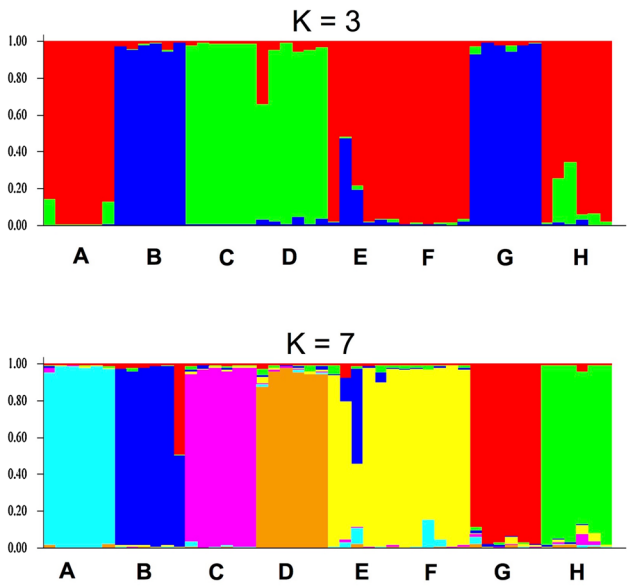


**Fig. 5** STRUCTURE plots at K = 3 and K = 7. *Key* A = Mazo (La Palma), B = Punta de Arenas Blancas (El Hierro), C = Tejina-Milán (Tenerife), D = Puntallana (La Gomera), E = Poris de Abona (Tenerife), F = Güimar (Tenerife), G = Tecorone (El Hierro), H = Barranco de Ruiz (Tenerife). (Color figure online)

separated from all the other Tenerife populations, which group together and with the La Palma population (A, E, F, H). The third group is formed by the El Hierro populations (B, G).

## Discussion

### The island pattern

Of the islands in which *L. sessilifolius* is distributed, Tenerife is the oldest (11.6 Ma) followed by La Gomera (10 Ma), La Palma (2 Ma) and El Hierro (1 Ma) (Carracedo 1994; Juan et al. 2000; Fernández-Palacios et al. 2011). Figure 3 shows clearly that when multiple populations were examined for an island, they generally clustered together (El Hierro: G, B and Tenerife: E, F, H). The only exception is the divergent population from Anaga, Tenerife (Tejina-Milán, C in Fig. 3), which is discussed below. These nuclear microsatellites do not reflect the morphological division of *L. sessilifolius* DC. into two subspecies (*L. sessilifolius* subsp. *villossisimus* from El Hierro and subsp. *sessilifolius* from other islands) (see Fig. 1).

Tenerife was originally composed of three palaeoislands: Adeje (11.6–3.5 Ma), Teno (6.7–4.5 Ma), and Anaga (6.5–3.5 Ma) (Ancochea et al. 1990; Fernández-Palacios et al. 2011). These palaeoislands were then joined into the modern island of Tenerife by volcanic activity, a process that was complete by c. 2 Ma (Ancochea et al. 1990), with connection between the palaeoislands likely achieved by 3.5 Ma. The paleoislands of Tenerife, particularly Teno and Anaga, are very rich in local endemics (Trusty et al. 2005), feasibly due to the isolation of these lineages followed by endemic evolution prior to 3.5 Ma. Furthermore, a series of massive landslips (c. 170–800 ka) may have helped to keep the palaeoislands isolated, even after connection (Ancochea et al. 1990; Watts and Masson 1995; Juan et al. 2000).

It is therefore not surprising that the Anaga population (C: Tejina-Milán) of *L. sessilifolius* is highly distinctive. Cluster analysis and STRUCTURE are consistent in dividing *L. sessilifolius* populations in two: Tejina-Milán vs the rest. One hypothesis that would be consistent with our results is that *L. sessilifolius* evolved in Anaga and La Gomera and then spread to other islands and back-colonized Tenerife. In this context it should be mentioned that Anaga and Gomera formed part of a palaeo-archipelago in the past (Fernandez-Palacios et al. 2011). *L. sessilifoliius* has also been reported from Teno and Adeje but samples from these areas are not included here. Given the distinctiveness of the Anaga population, future investigation of the Teno and Adeje populations is warranted.

### Gene flow between adjacent populations

It seems likely that considerable gene flow occurs between populations Poris de Abona (pop E) and Güimar (pop F). This is indicated by the intermixing of individuals from these two populations in the tree of Fig. 2, and the high diversity measures (including allelic richness) shown by

each of these populations in Table 3. Gene flow between them is likely, as they are located near each other on the southeastern coast of Tenerife with no physical barriers separating them.

### Tejina-Milán: a distinctive population with low genetic diversity in Tenerife

The Tejina-Milán population (Tenerife) is outstanding because of its high genetic differentiation with respect to the other Tenerife populations. In the UPGMA tree, it is sister to all other populations of *L. sessilifolius*. This distinctiveness of the Tejina-Milán population may indicate that this is an anciently diverged population. The origin of this distinct lineage may be explained by the geological history of the Anaga peninsula. The Anaga palaeoisland and La Gomera represent some of the oldest areas of the western Canary Islands (Carracedo and Day 2002). The topology of the UPGMA tree supports that the colonization sequence of the lineage of *L. sessilifolius* proceeded according to the ages of the geological substrates where it occurs, so that the other Tenerife populations would represent more recent colonization events, ultimately deriving from either La Gomera or Anaga. Previous dating of the diversification of the *L. sessilifolius* lineage (Ojeda et al. 2012) indicates that the group radiated within the last 2 Mya, around the time of the most recent volcanic activity in Tenerife.

The low genetic diversity of the Anaga population (population C) possibly indicates that this is a relictual population that has undergone a range contraction. Population C has a very restricted area and is isolated by surrounding populations of *Lotus tenellus*. Our findings are in contrast with the results in *Canarina canariensis* (L.) Vatke (Campanulaceae), which found higher genetic diversity on populations distributed on the paleo-islands of Anaga, Teno and Roque del Conde, suggesting that these populations likely represent ancestral lineages (Mairal et al. 2015). Within genus *Lotus* there are two other described species in the northeast side of Anaga (*Lotus dumetorum* and *L. tenellus*), but we have not observed hybrids between any of them and *L. sessilifolius*. In addition, with the exception of a single population of *L. tenellus*, we did not observe any populations of related species in the close vicinity of the Tejina-Milán population.

There are no obvious morphological distinctions between the Tejina-Milán and the other *L. sessilifolius* populations in Tenerife; however, if morphological characters were to be found distinguishing this population, then formal taxonomic recognition (for instance at subspecific level) should be considered. On the basis of the results presented here, especial conservation measures should be considered for this population. For instance it is important to maintain the distinctiveness of this population by avoiding the introduction of *L. sessilifolius* from other parts of the island to the Anaga peninsula Biosphere Reserve, and monitoring development so as not to allow the extinction of this population.

### General implications for conservation genetics

*Lotus sessilifolius* is one of the most common and variable of the endemic *Lotus* species of Macaronesia. However, as noted above, the microsatellite variation has revealed deep genetic structuring which should be used to inform conservation. The Anaga peninsula has many locally endemic plant taxa, which are interpreted as resulting from geological history, such as *Micromeria glomerata* and *M. rivas-martinezii* (Puppo et al. 2014, 2016). However, in *L. sessilifolius* the variation is cryptic in that there are no obvious phenotypic differences. It is possible that there are further distinct populations of this lineage, and others, on the Anaga Peninsula yet to be discovered. Additional genetic analysis, including from the other palaeoislands, Teno and Adeje, may therefore uncover more cryptic diversity in these regions.

In addition, the microsatellite primers described here are potentially of great value for conservation genetic studies of the many rare and endangered *Lotus* species of Macaronesia (Table 4). Initial tests suggest that the primers amplify successfully in all the species listed in Table 4. Many Canarian *Lotus* species have narrow distributions and are often restricted to specific habitats within each island. Thus, the group is highly susceptible to habitat destruction, and at least 11 species are listed under some category of threat, from rare to critically endangered (Table 4) (VV. AA. 2000; Bañares et al. 2004; Martín et al. 2008). Despite the relatively high number of endangered species, only two previous studies have assessed the genetic diversity in six endemic species (three endangered) using allozymes (Oliva-Tejera et al. 2005, 2006). Of the six species analyzed, only the endangered *L. kunkelii* showed relatively low levels of genetic variability, presumably due to inbreeding, very low population size and geographic isolation (Oliva-Tejera et al. 2006). The remaining species analyzed showed high levels of genetic variability, comparable to those reported in other continental *Lotus* species (Gauthier et al. 1998). The microsatellite tools reported here will facilitate conservation genetics work on diverse *Lotus* species.

**Table 4** Macaronesian *Lotus* species considered under different levels of threat, according to Red List of Spanish Vascular Flora based on the IUCN Red Data Book (IUCN) (VV. AA. 2000), the Atlas of Endangered Spanish Vascular Flora (AESVF) (Bañares et al. 2004), and the ranking according to the top 100 endangered species of Macaronesia (Martín et al. 2008)

| Species | IUCN 2000 | AESVF 2004 | Rank within the top 100 in Macaronesia | Distribution |
|---|---|---|---|---|
| Rhyncholotus group | | | | |
| *L. berthelotii* | CR | CR | 7 | Tenerife |
| *L. eremiticus* | CR | CR | 25 | La Palma |
| *L. maculatus* | CR | CR | 3 | Tenerife |
| *L. pyranthus* | CR | CR | 49 | La Palma |
| Section *Pedrosia* | | | | |
| *L. arinagensis* | CR | CR | – | Gran Canaria |
| *L. callis-viridis* | EN | EN | – | Gran Canaria |
| *L. dumetorum* | VU | – | – | Tenerife |
| *L. genistoides* | – | CR | – | Gran Canaria |
| *L. kunkelii* | CR | CR | 6 | Gran Canaria |
| *L. mascaensis* | VU | – | – | Tenerife |
| *L. spartioides* | – | VU | – | Gran Canaria |

Numbers indicate their rank under the top 100 list, – not considered within the 100 most endangered species

*CR* critically endangered, *EN* endangered, *VU* vulnerable

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