American Journal of Botany 93(8): 1116–1124. 2006.

PATTERNS OF GENETIC DIVERGENCE OF THREE CANARIAN ENDEMIC *LOTUS* (FABACEAE): IMPLICATIONS FOR THE CONSERVATION OF THE ENDANGERED *L. KUNKELII*[1]

FELICIA OLIVA-TEJERA,[2] JULI CAUJAPÉ-CASTELLS,[2,5] JOSEFA NAVARRO-DÉNIZ,[2] ALFREDO REYES-BETANCORT,[3] STEPHAN SCHOLZ,[4] MARIO BACCARANI-ROSAS,[2] AND NEREIDA CABRERA-GARCÍA[2]

[2]Laboratorio de Biodiversidad Molecular y Banco de ADN, Jardín Botánico Canario Viera y Clavijo, Ap. de correos 14 de Tafira Alta, 35017 Las Palmas de Gran Canaria, Spain; [3]Jardín de Aclimatación de La Orotava (ICIA), C. Retama 2, 38400 Puerto de la Cruz, Tenerife, Spain; and [4]Casa Sick-Esquinzo, 35626 Jandía, Fuerteventura, Spain

We examined data for 11 allozyme loci in 14 populations that represent the distribution of the endangered *Lotus kunkelii*, the narrowly distributed *L. arinagensis* (both endemic to Gran Canaria), and the broad-ranging *L. lancerottensis* (endemic to the easternmost Canary Islands, Fuerteventura and Lanzarote) to explore and construe patterns of genetic variation and use this data to assess the controversial taxonomic status of *L. kunkelii* relative to *L. lancerottensis*. While *L. kunkelii* maintains low levels of variation, presumably as a consequence of prolonged inbreeding due to very low population size and sharp geographic isolation, the other two taxa have much higher indicators of polymorphism than those reported for other oceanic island endemics. *Lotus arinagensis* has the highest genetic polymorphism and the lowest interpopulation differentiation, presumably because of its considerable antiquity and habitat stability, despite recent fragmentation. The high interpopulation differentiation in *L. lancerottensis* is attributed to the Atlantic acting as a barrier, reducing gene flow within islands. Evolutionary analysis of the allozyme evidence indicates that *L. kunkelii* is genetically closer to *L. arinagensis* than to *L. lancerottensis,* thereby dispelling the taxonomic uncertainty and supporting *L. kunkelii* as a distinct species, warranting legal protection in the forthcoming catalog of threatened Canarian species.

Key words: allozymes; Canary Islands; conservation; endemics; evolutionarily significant units; genetic divergence; *Lotus*; taxonomy.

Morphological characters offer variation that has proven useful to assess evolutionary relationships or to establish unambiguous taxonomic circumscriptions in most plant lineages. However, this variation is difficult to handle statistically because it is mostly attributable to the combined action of many different loci (Doebley et al., 1990; Koornneef et al., 2004), whose inheritance and exact contribution to the phenotype are unknown. In addition, environmentally induced variation may be confused easily with genetically based (i.e., systematically meaningful) variation, and this difficulty may sometimes undermine the value of evolutionary or taxonomic inferences based only on morphological traits. These intrinsic limitations notwithstanding, when morphology cannot provide unambiguous diagnostic characters, the taxonomist is hindered by lack of useful characters, thereby begetting controversies that make the delimitation of operative taxonomic units (OTUs) difficult. In these cases, it is of utmost importance to add variables other than the morphological to facilitate an objective classification, especially when the organisms are endangered and their taxonomic rank may affect their legal protection status.

Lotus kunkelii (Esteve) Bramwell & Davis and *L. lancerottensis* Webb & Berthel. are two taxa of *Lotus* sect. *Pedrosia* endemic to the eastern Canary Islands. While *L. kunkelii* is known from only one extremely small population (less than 50 individuals according to Bañares et al., 2003) on the eastern coast of the island of Gran Canaria, *L. lancerottensis* is widespread on Fuerteventura and Lanzarote, where it shows considerable ecological variation (Fig. 1). The taxonomic position of *L. kunkelii* and *L. lancerottensis* has stirred controversy ever since the former taxon was described by Esteve-Chueca (1972). This author treated *L. kunkelii* as a subspecies of *L. lancerottensis* (*L. lancerottensis* subsp. *kunkelii* Esteve) based on several morphological traits. However, Bramwell and Davis (1972) contend that *L. kunkelii* should be treated as a distinct species. Because no other data have been examined for these two taxa until now, the taxonomic status dispute has remained unsettled.

This issue has become important from a conservation standpoint because the new methodology for the catalog of threatened Canarian species (Martín-Esquivel, 2004) argues that the threshold between subspecific and interpopulation differentiation is ambiguous, and establishes that the minimum unit for legal protection should be the species. Although this is an arbitrary decision not supported by science, solving the taxonomic dispute that affects *L. kunkelii* is of clear conservation interest because its consideration as a species or as a subspecies of *L. lancerottensis* will determine, respectively, its inclusion in the lists of protected Canarian

[1] Manuscript received 2 September 2005; revision accepted 6 May 2006.

The authors thank D. Bramwell, B. Navarro, and J. Naranjo (at the Jardín Botánico Canario "Viera y Clavijo"); J. R. Acebes-Ginovés (at the Departamento de Botánica of the Facultad de Farmacia in the Universidad de La Laguna, Tenerife) for comments and insight on an earlier version of this manuscript; staff of BIOTA-genes at the Centro de Planificación Ambiental of the Gobierno de Canarias in Tenerife for their trust and stimulus; and the Cabildo de Gran Canaria for institutional support. This investigation was funded by the European initiative INTERREG IIIB-Atlántico and the Gobierno de Canarias to the project BIOTA-genes. This paper was written while J. C. C. was a "Ramón y Cajal" researcher in molecular population genetics and phylogenetics at the JBCVC. J. C. C. thanks the Ministerio de Educación y Ciencia and the Cabildo de Gran Canaria for their support.

[5] Author for correspondence (e-mail: julicaujape@grancanaria.com)

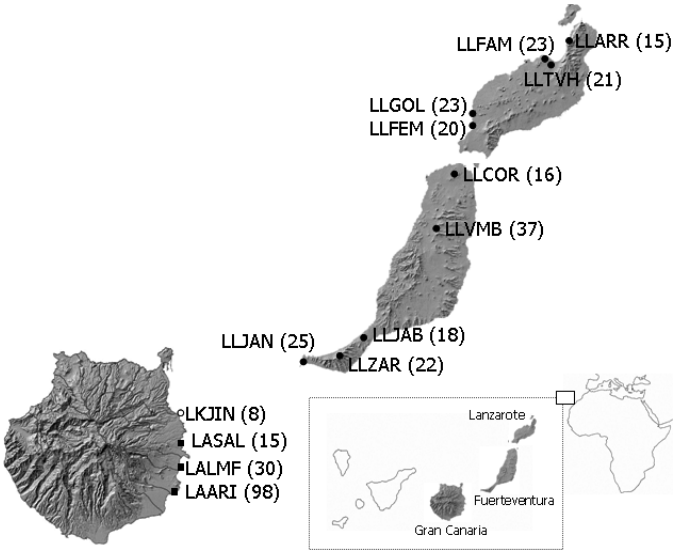

Fig. 1. Distribution map of the 14 populations of *L. arinagensis* (black squares), *L. kunkelii* (white circle), and *L. lancerottensis* (black circles) sampled in the Canary Islands for this investigation. Numbers in parentheses are sample sizes. Population codes: *L. kunkelii* = LKJIN (Barranco de Jinámar); *L. lancerottensis* = LLFEM (Femés), LLGOL (Montaña del Golfo), LLARR (Arrieta-Orzola), LLFAM (Caleta de Famara), LLTVH (Teguise-Los Valles-Haría), LLCORR (road to Corralejo), LLVMB (Villaverde-La Matilla-Betancuria), LLJAB (El Jable), LLZAR (Pico de la Zarza), LLJAN (Punta de Jandía); *L. arinagensis* = LAARI (Playa de Arinaga), LALMF (Playa de la Mar Fea), LASAL (Las Salinetas). Voucher specimens are deposited in the herbarium LPA.

endemics or its exclusion from this instrument of legal protection.

The area where this population lives was first protected by the Canarian Environmental Law under the designation of "natural landscape of interest" (Boletín Oficial de Canarias, 1987), which was later renamed as a "site of scientific interest" (Boletín Oficial de Canarias, 1994). Despite this legal protection, recent anthropogenic pressures in the area of distribution of *L. kunkelii* have brought about enhanced habitat degradation and a swift decline in the number of individuals, which greatly increase the threat of endangerment.

The discrete data on genetic variation afforded by allozymes are more amenable to mathematical analysis than are morphological traits and have often been used with success as surrogates for quantitative genetic variation. Although in recent meta-analyses (Reed and Frankham, 2001) a low correlation between molecular and quantitative variables has been found, allozymes have so far provided valuable ancillary information to clarify taxonomic issues in a number of plant lineages (Elisiário et al., 1999; Cabrita et al., 2001), including other Canarian *Lotus* within sect. Pedrosia (Oliva-Tejera et al., 2005). Therefore, we consider this molecular technique a proper first choice to address the taxonomic issue that affects *L. kunkelii* and *L. lancerottensis* so that we can understand their pattern of genetic divergence. This approach will set the stage for improving our knowledge of the evolution and taxonomy of the group.

Our specific objectives in this investigation were (1) to explore and construe the patterns of evolutionary divergence in *L. kunkelii* and *L. lancerottensis* using allozyme variation and (2) to use this molecular information as a tool for assessing the protection status of *L. kunkelii*. To gauge the extent of genetic differences between *L. kunkelii* and *L. lancerottensis*, we include *L. arinagensis* Bramwell (a well-delimited species within sect. *Pedrosia* that is distributed exclusively along the eastern coast of Gran Canaria) as an external reference.

MATERIALS AND METHODS

Plant material—*Lotus lancerottensis* ($2n = 14$; Larsen, 1956) is a perennial plant covered with silky appressed hairs. Its stems are prostrate or ascendant, subglabrous, with sessile five-foliolate leaves. The leaflets are obcordate to obovate, very rarely succulent. Inflorescences consist of peduncles of 2–4 cm each, the teeth are always longer than the tube, and legumes are smooth. By contrast, *L. kunkelii* ($2n = 28$, Ortega, 1976) possesses a densely hirsute stem with patent hairs, and its leaves are densely hairy and much smaller than those of *L. lancerottensis*. The leaflets are always succulent, obcordate to rounded, the calyx teeth are the same length as the tube or shorter, and the legumes are rough. *Lotus arinagensis* [$2n = 28$; Ortega, 1976, as *L. leptophyllus* (Lowe) K. Larsen] is a slow-growing perennial, with densely tomentose (white) prostrate stems. Its leaves are sometimes three-foliolate, and leaflets are ovoid, silvery, and subsucculent. The inflorescences have peduncles of 1–1.5 cm, and legumes are smooth.

Sampling—In all cases, the sampling design followed Caujapé-Castells (2004) and included all the known areas of population distribution in order to estimate consistently the levels of genetic variation. The only known population of the endangered *L. kunkelii* occurs at the Barranco de Jinámar (Gran Canaria), and its estimated size is less than 50 individuals according to the latest census (Bañares et al., 2003). In this specific case, sampling was restricted to the individuals whose size was enough to ensure plant survival after leaf detachment. In the broad-ranging *L. lancerottensis*, we sampled 220 individuals from 10 populations (five from Lanzarote and five from Fuerteventura). In the narrowly distributed *L. arinagensis*, we sampled 143 individuals from the three known populations in Gran Canaria. Sample sizes (Fig. 1) are strictly related to the size of target populations and ranged from eight individuals in population LKJIN (*L. kunkelii*) to 98 in LAARI (*L. arinagensis*). In all cases, leaf samples from individual plants were deposited in numbered, zippered plastic bags that were then refrigerated in a portable cooler until storage in ultralow freezers at the Jardín Botánico Canario "Viera y Clavijo" (JBCVC) in Gran Canaria, where they remained until used for extract preparation. The time from leaf collection to storage in the ultralow freezers was under 4 d in all cases; longer times would have entailed a loss of activity for most assayed enzymes (F. Oliva-Tejera, Jardín Botánico Canario "Viera y Clavijo", unpublished data).

Electrophoretic analysis and data processing—Allozyme electrophoreses and interpretations followed, respectively, Caujapé-Castells et al. (2001) and Oliva-Tejera et al. (2005) and allowed us to score six enzymes: phosphoglucomutase (PGM, E.C. 5.4.2.2), isocitrate dehydrogenase (IDH, E.C.1.1.1.42), esterase (EST, 3.1.1.1), phosphogluconate dehydrogenase (6PGD, E.C.1.1.1.44), malate dehydrogenase (MDH, E.C.1.1.1.37), and malic enzyme (ME, E.C.1.1.1.40). All gel interpretations were drawn in the matrix provided by the computer program Transformer-3 (Caujapé-Castells and Baccarani-Rosas, 2005), which was also used to generate all the input files needed to analyze these data using different population genetic softwares.

Number of alleles per locus (A), percentage of polymorphic loci (P) [a locus was considered polymorphic if more than one allele was detected], H_o (i.e., average heterozygosity based on direct counts), H_e (i.e., average proportion of heterozygotes based upon Hardy–Weinberg expectations), inbreeding coefficients [$F_{IS} = 1 - (H_o/H_e)$] (Hartl and Clark, 1989), and genetic identities (I_{NEI}; Nei, 1978) were calculated using BIOSYS-1, version 1.7 (Swofford and Selander, 1989) at the species and population levels from genotype data corresponding to each locus. I_{NEI} was calculated for comparative purposes with other studies with congeneric Canarian endemics that use this index to quantify the extent of genetic divergence. Popgene, version 1.32 (Yeh et al. 1997), was used to calculate the average effective number of alleles per locus (A_e) following the formula $A_e = 1 + 4N_e\mu$ (Kimura and Crow, 1964) and to carry out Ewens–Watterson homozygosity tests of neutrality (Watterson, 1978).

The values of Rogers' genetic distance (Rogers, 1972) between population pairs were used to build a cluster with the neighbor joining (NJ) algorithm (Fig. 2). Rogers' distance is more sensitive to disjunct or private alleles than other

TABLE 1. Allele frequencies at the 11 loci interpreted in the 14 populations of *Lotus kunkelii*, *L. lancerottensis*, and *L. arinagensis* in the Canary Islands. Integer numbers are the individuals scored per locus and per population. Framed frequencies signal the population distribution of taxon-exclusive alleles, and gray frequencies are the alleles exclusive either to Fuerteventura or Lanzarote (considering only the populations of *L. lancerottensis*). Population codes correspond to Fig. 1.

	L. kunkelii	*L. lancerottensis*										*L. arinagensis*		
	Gran Canaria	Lanzarote					Fuerteventura					Gran Canaria		
Locus/allele	LKJIN	LLFEM	LLGOL	LLARR	LLFAM	LLTVH	LLCOR	LLVMB	LLJAB	LLZAR	LLJAN	LAARI	LALMF	LASAL
Idh-1	7	20	21	14	18	21	15	37	13	22	25	98	30	9
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000
b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.050	0.000
c	0.286	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.097	0.083	0.111
d	0.500	1.000	0.976	1.000	0.972	1.000	1.000	1.000	1.000	0.841	0.980	0.857	0.850	0.889
e	0.214	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
f	0.000	0.000	0.024	0.000	0.028	0.000	0.000	0.000	0.000	0.159	0.020	0.020	0.000	0.000
Est-1	8	17	14	13	20	20	15	31	15	18	23	73	26	10
a	0.000	0.000	0.036	0.039	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	0.375	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000
c	0.250	0.000	0.000	0.000	0.025	0.000	0.000	0.065	0.133	0.000	0.044	0.240	0.000	0.100
d	0.000	0.000	0.000	0.000	0.050	0.075	0.200	0.226	0.100	0.194	0.174	0.206	0.039	0.000
e	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000
f	0.313	0.706	0.643	0.000	0.225	0.100	0.233	0.532	0.567	0.722	0.783	0.534	0.769	0.900
g	0.000	0.294	0.321	0.962	0.700	0.825	0.567	0.177	0.133	0.083	0.000	0.007	0.135	0.000
h	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.067	0.000	0.000	0.000	0.058	0.000
Pgm-1	8	20	22	15	23	21	15	37	18	22	25	97	29	15
a	0.000	0.025	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.023	0.040	0.016	0.035	0.100
b	1.000	0.975	1.000	1.000	0.978	1.000	1.000	1.000	1.000	0.977	0.960	0.984	0.966	0.900
Pgm-2	8	20	21	15	23	21	15	36	18	22	25	98	29	15
a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.041	0.000	0.133
b	0.000	0.000	0.000	0.000	0.000	0.000	0.067	0.083	0.000	0.000	0.000	0.000	0.000	0.000
c	1.000	0.975	1.000	0.933	1.000	0.929	0.867	0.917	1.000	1.000	0.960	0.954	1.000	0.867
d	0.000	0.025	0.000	0.067	0.000	0.071	0.067	0.000	0.000	0.000	0.040	0.005	0.000	0.000
Pgm-3	6	13	21	13	14	21	13	37	15	19	24	98	30	14
a	0.000	0.000	0.000	0.000	0.107	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100	0.179
c	0.667	0.923	0.691	0.346	0.179	0.500	0.500	0.473	0.467	0.421	0.458	0.801	0.883	0.714
d	0.250	0.077	0.310	0.654	0.714	0.500	0.500	0.514	0.500	0.579	0.521	0.194	0.017	0.107
e	0.083	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.033	0.000	0.021	0.005	0.000	0.000
6-Pgd-1	8	19	16	12	21	19	12	36	18	20	25	98	30	15
a	1.000	0.816	0.875	1.000	0.833	0.842	0.917	0.944	0.972	0.900	1.000	0.980	0.900	1.000
b	0.000	0.184	0.125	0.000	0.167	0.158	0.083	0.056	0.028	0.100	0.000	0.020	0.100	0.000
6-Pgd-2	8	18	13	12	20	21	11	36	18	22	25	98	30	15
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.986	1.000	1.000	1.000	1.000	1.000	1.000
b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000
6-Pgd-3	3	18	13	11	11	15	12	33	17	20	23	98	28	9
a	0.667	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.393	0.000
b	0.333	0.111	0.231	0.091	0.091	0.133	0.125	0.515	0.000	0.175	0.609	0.378	0.446	0.778
c	0.000	0.861	0.692	0.818	0.864	0.800	0.792	0.470	0.912	0.725	0.391	0.555	0.161	0.222
d	0.000	0.028	0.077	0.091	0.046	0.067	0.083	0.015	0.088	0.100	0.000	0.043	0.000	0.000
6-Pgd-4	5	14	8	12	19	12	11	33	17	14	25	58	22	3
a	0.000	0.321	0.563	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.043	0.023	0.000
b	1.000	0.679	0.438	0.917	0.211	0.458	0.546	0.530	0.794	0.357	0.820	0.362	0.182	0.667
c	0.000	0.000	0.000	0.042	0.447	0.458	0.091	0.000	0.029	0.143	0.020	0.328	0.455	0.333
d	0.000	0.000	0.000	0.042	0.342	0.083	0.364	0.470	0.177	0.500	0.160	0.250	0.341	0.000
e	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000

TABLE 1. Continued.

	L. kunkelii	*L. lancerottensis*										*L. arinagensis*		
	Gran Canaria	Lanzarote					Fuerteventura					Gran Canaria		
Locus/allele	LKJIN	LLFEM	LLGOL	LLARR	LLFAM	LLTVH	LLCOR	LLVMB	LLJAB	LLZAR	LLJAN	LAARI	LALMF	LASAL
Mdh-1	8	19	22	14	23	21	14	34	18	21	25	94	29	9
a	0.000	0.632	0.023	0.357	0.152	0.262	0.071	0.324	0.000	0.238	0.020	0.053	0.035	0.000
b	0.000	0.211	0.364	0.214	0.022	0.429	0.500	0.309	0.222	0.191	0.000	0.181	0.172	0.333
c	0.750	0.158	0.591	0.357	0.435	0.143	0.429	0.368	0.778	0.571	0.480	0.729	0.552	0.667
d	0.250	0.000	0.023	0.000	0.065	0.167	0.000	0.000	0.000	0.000	0.020	0.027	0.241	0.000
e	0.000	0.000	0.000	0.071	0.326	0.000	0.000	0.000	0.000	0.000	0.480	0.011	0.000	0.000
Mdh-2	8	19	22	14	23	21	14	34	18	21	25	94	29	9
a	0.357	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.035	0.000
b	0.000	0.028	0.000	0.067	0.261	0.000	0.094	0.097	0.000	0.068	0.020	0.011	0.000	0.333
c	0.643	0.944	1.000	0.933	0.739	1.000	0.906	0.903	0.972	0.932	0.900	0.974	0.948	0.667
d	0.000	0.028	0.000	0.000	0.000	0.000	0.000	0.000	0.028	0.000	0.080	0.011	0.017	0.000

measures of genetic distance that use an overall allele frequency; therefore, it is generally believed to be more appropriate to address cases of presumably recent evolutionary divergence (Rogers, 1991; Britten and Brussard, 1992). The NJ algorithm allows for unequal rates of molecular evolution and is thus more likely to reflect evolutionary relationships than other clustering methods.

Nei's (1973) population-structure statistics and Wright's (1951) *F* statistics were calculated over all loci for the three taxa using GeneStat-PC 3.31 (Lewis and Whitkus, 1993) and BIOSYS-1, version 1.7 (Swofford and Selander, 1989). To evaluate the genetic differences among *L. kunkelii* and the other two taxa, *F* statistics were also calculated for the artificial assemblages (*L. kunkelii* + *L. arinagensis*, *L. kunkelii* + *L. lancerottensis* and *L. arinagensis* + *L. lancerottensis*). The rationale of this procedure is to use the eventual changes in the values of F_{ST} to assess whether the addition of the population of *L. kunkelii* disrupts significantly the genetic cohesion of *L. lancerottensis* (in which case *L. kunkelii* should be considered a species on its own) or not (and then it would be feasible to consider *L. kunkelii* as a disjunct population of *L. lancerottensis*). The values of F_{ST} for *L. arinagensis* alone and in combination with the other two taxa were used to calibrate the results obtained for *L. kunkelii* and *L. lancerottensis*.

We applied a sign test for heterozygosity excess (Cornuet and Luikart, 1996) to detect whether populations had experienced recent historical bottlenecks. This test compares expected heterozygosity (H_e) under Hardy–Weinberg equilibrium to the heterozygosity expected at mutation–drift equilibrium (H_{eq}) in a sample that has the same size and the same number of alleles as the sample used to measure H_e (Luikart and Cornuet, 1998). Because low frequency alleles are lost at a much faster rate than heterozygosity in a bottleneck situation, bottlenecked populations are expected to have a heterozygote excess. Calculations were made based on allele frequency data under the Stepwise Mutation Model (SMM) and the Independent Allele Model (IAM) using the program Bottleneck-PC (Piry et al., 1998).

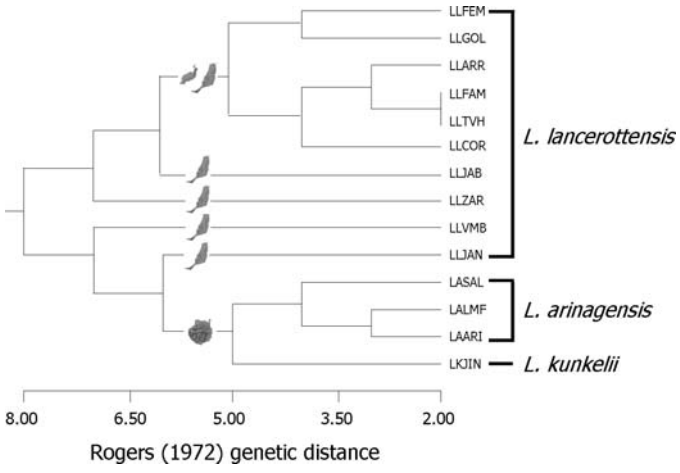

Fig. 2. Neighbor-joining cluster of the 14 populations of Canarian endemic *Lotus* based on Rogers (1972) genetic distance between population pairs. The island silhouettes indicate the distribution of each resulting group. Population codes correspond to Fig. 1.

RESULTS

Genetic interpretations of the six enzymes resolved allowed us to score 11 putative loci, none of which was monomorphic throughout the populations. Of the 47 alleles scored (Table 1), one was exclusive to the endangered *L. kunkelii* (*Idh1-e*), four to the broad-ranging *L. lancerottensis* (*Est1-a* and *Pgm3-a* to Lanzarote, and *Pgm2-b* and *6Pgd2-b* to Fuerteventura), and five to the narrowly distributed *L. arinagensis* (*Idh1-a*, *Idh1-b*, *Pgm2-a*, *Pgm3-b*, *6Pgd4-e*) (Table 1). We detected two alleles shared exclusively between *L. kunkelii* and *L. arinagensis* (*Est1-b*, *Est1-e*), one between *L. arinagensis* and *L. lancerottensis* (*Est1-h*) and none between *L. kunkelii* and *L. lancerottensis*. The remaining 34 alleles were shared by different combinations of the 14 populations analyzed. We did not detect diagnostic alleles (i.e., alleles that were monomorphic in one taxon and not present in the others).

The means of the basic indicators of polymorphism (Table 2) showed a remarkable uniformity across taxa, with minimum values of *A* and *P* in *L. kunkelii* ($A = 1.9$, $P = 54.5$) and maximum values in *L. arinagensis* ($A = 2.7$, $P = 69.7$). Mean observed and expected heterozygosities ranged from $H_o = 0.113$ in *L. kunkelii* to $H_o = 0.143$ in *L. lancerottensis* and from $H_e = 0.254$ in *L. lancerottensis* to $H_e = 0.306$ in *L. kunkelii*. Fixation indices (F_{IS}) spanned from $F_{IS} = 0.342$ (*L. lancerottensis*) to $F_{IS} = 0.646$ (*L. kunkelii*). At the population level, the average coefficients of inbreeding (Table 2) were all moderately to highly positive, with the highest ones detected in *L. kunkelii* (LKJIN, $F_{IS} = 0.646$) and in one population of *L. arinagensis* (LASAL, $F_{IS} = 0.677$). None of the examined populations showed evidence of a recent bottleneck, and all loci behaved as neutral according to Ewens–Watterson homozygosity test (Table 3).

Values of F_{ST} and G_{ST} (Table 3) are moderately low in the two taxa that have more than one extant population. F_{ST} of 0.090 in *L. arinagensis* is roughly half the value estimated for *L. lancerottensis* sensu lato ($F_{ST} = 0.182$). Values of F_{ST} for

TABLE 2. Basic indicators of isozyme variability for the 14 populations of *Lotus* surveyed in the Canary Islands. T = total number of alleles scored; A_l = mean number of alleles per locus; P = proportion of polymorphic loci (a locus is regarded as polymorphic when the frequency of the most common allele does not exceed 0.95); H_o and H_e = observed and expected heterozygosities, respectively; F_{IS} = fixation index. Values in parentheses are standard deviations.

								Bottleneck tests				
									IAM		SMM	
Population	*T*	A_l	A_e	*P*	H_o	H_e	F_{IS}	PL	*Hd/He*	*P*	*Hd/He*	*P*
L. kunkelii												
1. LKJIN	21	1.9 (0.3)	1.4 (0.5)	54.5	0.113 (0.060)	0.306 (0.092)	0.646	5	1/4	0.625	2/3	1.000
L. lancerottensis												
2. LLFEM	23	2.1 (0.2)	1.3 (0.4)	63.6	0.050 (0.015)	0.213 (0.059)	0.473	9	5/4	0.820	5/4	0.203
3. LLGOL	22	2.0 (0.3)	1.5 (0.5)	54.5	0.096 (0.056)	0.250 (0.074)	0.535	7	2/5	0.109	5/2	0.813
4. LLARR	22	2.0 (0.3)	1.4 (0.7)	54.5	0.106 (0.062)	0.183 (0.070)	0.321	7	5/2	0.688	5/2	0.039
5. LLFAM	28	2.5 (0.4)	1.7 (0.8)	63.6	0.151 (0.038)	0.301 (0.077)	0.346	9	4/5	0.734	5/4	0.359
6. LLTVH	23	2.1 (0.3)	1.6 (0.7)	63.6	0.163 (0.092)	0.263 (0.079)	0.422	8	3/5	0.250	3/5	0.742
7. LLCOR	24	2.2 (0.3)	1.6 (0.6)	72.7	0.139 (0.089)	0.295 (0.075)	0.591	8	4/4	0.641	4/4	0.547
8. LLVMB	25	2.3 (0.3)	1.7 (0.7)	72.7	0.174 (0.087)	0.303 (0.081)	0.428	9	4/5	0.250	4/5	1.000
9. LLJAB	23	2.1 (0.4)	1.4 (0.6)	45.5	0.163 (0.091)	0.198 (0.072)	0.184	7	4/3	0.578	5/2	0.039
10. LLZAR	24	2.2 (0.2)	1.5 (0.6)	72.7	0.205 (0.069)	0.295 (0.071)	0.264	8	2/6	0.055	4/4	0.844
11. LLJAN	26	2.4 (0.3)	1.4 (0.4)	54.5	0.185 (0.078)	0.238 (0.065)	0.201	9	6/3	0.652	7/2	0.129
Mean *L. lancerottensis*		2.2 (0.3)	1.5 (0.6)	61.8	0.143 (0.068)	0.254 (0.072)	0.342	—	—	—	—	—
L. arinagensis												
12. LAARI	39	3.5 (0.5)	1.6 (0.8)	54.5	0.193 (0.055)	0.282 (0.079)	0.183	10	6/4	0.695	9/1	0.003
13. LALMF	31	2.8 (0.4)	1.6 (0.7)	72.7	0.134 (0.037)	0.285 (0.076)	0.391	9	6/3	0.910	6/3	0.129
14. LASAL	21	1.9 (0.2)	1.4 (0.3)	81.8	0.068 (0.036)	0.284 (0.057)	0.677	9	4/5	0.203	4/5	0.910
Mean *Lotus arinagensis*		2.7 (0.4)	1.5 (0.6)	69.7	0.132 (0.043)	0.284 (0.071)	0.417	—	—	—	—	—
Total means		2.3 (0.3)	1.5 (0.6)	63.0	0.139 (0.062)	0.264 (0.073)		—	—	—	—	—

populations of *L. lancerottensis* were similarly high (F_{ST} = 0.177 for Fuerteventura and F_{ST} = 0.181 for Lanzarote). The lowest I_{NEI} between populations (I_{NEI} = 0.748) corresponded to *L. kunkelii* compared with LLFAM (*Lotus lancerottensis* from Lanzarote), and the highest (I_{NEI} = 0.981) to a subset of populations of *L. lancerottensis* from Fuerteventura and Lanzarote (LLCOR-LLTVH, respectively) and a different subset from only Fuerteventura (LLVMB-LLZAR) (Table 4). The NJ cluster with Rogers (1972) genetic distance resulted in a single tree (Fig. 2), that placed *L. kunkelii* closer to *L. arinagensis* than to *L. lancerottensis*. However, two populations of *L. lancerottensis* from Fuerteventura (LLVMB and LLJAN) clustered with the populations of *L. arinagensis* and *L. kunkelii*.

Comparatively, the values of I_{NEI} fitted those calculated by Oliva-Tejera et al. (2005) for other Canarian endemic taxa of *Lotus*, but any artificial assemblage with *L. kunkelii* resulted in I_{NEI} far lower than expected for conspecific taxa according to that paper. The values of F_{ST} also increased substantially when *L. kunkelii* was added to form artificial assemblages with the other two taxa; while the highest F_{ST} was detected in *L. lancerottensis* + *L. kunkelii* (F_{ST} = 0.215, vs. F_{ST} = 0.189 for *L. lancerottensis* alone), the highest increase in the value of this parameter corresponded to *L. arinagensis* + *L. kunkelii* (F_{ST} = 0.162, vs. F_{ST} = 0.083 for *L. arinagensis* alone).

DISCUSSION

Levels and apportionment of genetic variation—These taxa of *Lotus* maintain substantial levels of genetic heterogeneity, consistent with the emerging picture of higher variation in Canarian endemics relative to those from other oceanic archipelagos (Francisco-Ortega et al., 2000). Their basic indicators of polymorphism (Table 2) are in all cases higher than the average values calculated by Hamrick and Godt (1989) for endemic plants (A = 1.39, P = 26.3, H_e = 0.063). However, Helenurm (2001) detected much higher levels of polymorphism in *Jepsonia malvaefolia* Small (Saxifragaceae), an endemic to the Channel Islands of California and Guadalupe Island (Mexico), with averages of A = 2.9, P = 95.2, H_e = 0.179. Overall, the values for the *Lotus* species analyzed in this paper are slightly higher than those reported for the Gran Canarian pine forest endemics *L. holosericeus* Webb & Berthel. and *L. spartioides* Webb & Berthel. (Oliva-Tejera et al., 2005). The endangered *L. kunkelii* also contains more variation than some populations analyzed by Oliva-Tejera et al. (2005). The values of these parameters for the broad-ranging *L. lancerottensis* and the narrowly distributed *L. arinagensis* are only slightly lower than those detected by Gauthier et al. (1998) in the mainland diploid species *L. alpinus* (Schleich. ex DC) Ramond (averages A = 2.8, H_o = 0.219, H_e = 0.279). The degree of genetic variation within these taxa, as measured by the average population diversity (H_s, Table 3), is in all cases much higher than the averages published for plants from Hawaii (H_s = 0.064, DeJoode and Wendel, 1992) or the Juan Fernández islands (H_s = 0.042, Crawford et al., 2001), and almost two-fold the average value published for Canarian taxa (H_s = 0.137, Francisco-Ortega et al., 2000).

The skew in sample sizes associated with the increasing rarity of populations and taxa is one additional factor that may have affected the probability of finding genetic heterogeneity, thereby decreasing the values of the indicators of genetic variation in small populations. While it is important to consider

TABLE 3. Ewens–Watterson (E-W) neutrality tests and population structure statistics following Nei (1973) and Wright (1951) for the polymorphic loci found in *Lotus lancerottensis and L. arinagensis*. SD = standard deviation; L95 = lower limit of the 95% confidence interval for the mean value of the E-W statistic; U95: = upper limit of the 95% confidence interval for the mean value of the E-W statistic. A locus can be regarded as neutral when its mean E-W value falls within the confidence interval.

	Multilocus structure statistics					Population structure statistics					
	Hardy–Weinberg equilibrium		E-W neutrality tests			Nei's (1973) unmodified			Wright's *F* statistics		
Taxon/Locus	χ^2	*p*	Mean	L95	U95	*Hs*	*Dst*	*Gst*	*Fis*	*Fit*	*Fst*
L. lancerottensis											
Idh-1	0.1	0.735	0.852	0.505	0.995	0.041	0.004	0.096	−0.133	−0.024	0.096
Est-1	539.9	0.000	0.511	0.253	0.902	0.436	0.184	0.297	0.501	0.649	0.297
Pgm-1	42.3	0.000	0.842	0.501	0.995	0.021	0.000	0.019	0.346	0.358	0.019
Pgm-2	172.9	0.000	0.733	0.364	0.991	0.078	0.004	0.046	0.527	0.549	0.046
Pgm-3	186.6	0.000	0.628	0.336	0.967	0.452	0.065	0.126	−0.601	−0.412	0.118
6Pgd-1	144.8	0.000	0.856	0.506	0.995	0.156	0.008	0.051	0.836	0.844	0.051
6Pgd-2	0.0	1.000	0.853	0.506	0.995	0.003	0.000	0.013	−0.014	−0.001	0.013
6Pgd-3	147.2	0.000	0.720	0.368	0.989	0.354	0.063	0.151	0.644	0.697	0.151
6Pgd-4	237.9	0.000	0.635	0.332	0.969	0.460	0.141	0.235	0.491	0.608	0.231
Mdh-1	638.3	0.000	0.571	0.299	0.940	0.581	0.121	0.173	0.911	0.926	0.173
Me-1	125.7	0.000	0.636	0.331	0.959	0.133	0.011	0.078	0.543	0.659	0.253
Total means *L. lancerottensis*						0.247	0.055	0.182	0.436	0.543	0.189
L. arinagensis											
Idh-1	52.4	0.000	0.549	0.281	0.909	0.240	0.001	0.004	0.319	0.322	0.005
Est-1	122.1	0.000	0.477	0.245	0.835	0.393	0.045	0.103	0.338	0.408	0.105
Pgm-1	0.1	0.746	0.839	0.505	0.993	0.092	0.003	0.028	−0.083	−0.053	0.028
Pgm-2	0.3	0.960	0.719	0.393	0.986	0.106	0.006	0.055	−0.123	−0.062	0.055
Pgm-3	75.7	0.000	0.619	0.331	0.958	0.326	0.015	0.045	0.213	0.251	0.048
6Pgd-1	0.2	0.673	0.836	0.504	0.993	0.073	0.004	0.048	−0.095	−0.042	0.048
6Pgd-3	108.6	0.000	0.612	0.335	0.950	0.504	0.093	0.156	0.678	0.721	0.133
6Pgd-4	216.6	0.000	0.523	0.274	0.884	0.595	0.065	0.098	0.561	0.604	0.098
Mdh-1	362.1	0.000	0.540	0.279	0.912	0.495	0.023	0.045	0.950	0.952	0.044
Me-1	123.7	0.000	0.633	0.325	0.958	0.198	0.044	0.180	0.814	0.833	0.104
Total means *L. arinagensis*						0.302	0.030	0.076	0.357	0.393	0.067

this aspect for the thorough discussion of results, it does not seem to preclude our findings. The populations LALMF and LASAL (*L. arinagensis*), that are about the same size as that of the endangered *L. kunkelii*, have indicators of genetic polymorphism much higher than *L. kunkelii* (Table 2). Therefore, the levels of genetic variation detected in these extremely small populations of *L. arinagensis* and *L. kunkelii* seem to be related more to the contrasting historical and biological features of the corresponding taxa than to their low sample size.

Lack of evidence for genetic bottlenecks in the three taxa analyzed (Table 2) and the positive F_{IS} values (Table 3) suggest that these high levels of genetic variation have been attained in a context of environmental stability and despite an overall predominance of inbreeding. As hypothesized by

TABLE 4. Nei's (1978) distances (bottom) and identities (top) for all possible pairwise combinations of the 14 sampled populations of *Lotus* species. Population codes correspond to Table 2.

	L. kunkelii	*L. lancerottensis*										*L. arinagensis*		
	Gran Canaria	Lanzarote					Fuerteventura					Gran Canaria		
Populations	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. LKJIN	—	0.808	0.845	0.802	0.748	0.770	0.820	0.854	0.868	0.831	0.884	0.885	0.883	0.891
2. LLFEM	0.214	—	0.959	0.896	0.854	0.914	0.923	0.926	0.924	0.920	0.891	0.915	0.882	0.882
3. LLGOL	0.168	0.042	—	0.901	0.893	0.921	0.956	0.944	0.963	0.952	0.924	0.956	0.918	0.921
4. LLARR	0.220	0.109	0.104	—	0.940	0.972	0.970	0.910	0.926	0.890	0.868	0.854	0.785	0.802
5. LLFAM	0.290	0.157	0.113	0.062	—	0.957	0.956	0.910	0.914	0.935	0.872	0.882	0.833	0.808
6. LLTVH	0.261	0.090	0.082	0.029	0.044	—	0.981	0.920	0.913	0.910	0.853	0.892	0.854	0.824
7. LLCOR	0.199	0.080	0.045	0.030	0.045	0.019	—	0.970	0.970	0.964	0.908	0.937	0.879	0.874
8. LLVMB	0.158	0.077	0.057	0.094	0.094	0.084	0.031	—	0.951	0.981	0.960	0.956	0.918	0.931
9. LLJAB	0.141	0.079	0.038	0.077	0.090	0.091	0.030	0.050	—	0.975	0.942	0.956	0.880	0.903
10. LLZAR	0.185	0.083	0.050	0.116	0.068	0.094	0.037	0.019	0.026	—	0.943	0.964	0.918	0.906
11. LLJAN	0.123	0.115	0.079	0.142	0.137	0.159	0.097	0.041	0.059	0.058	—	0.939	0.901	0.948
12. LAARI	0.122	0.088	0.045	0.157	0.126	0.115	0.065	0.045	0.045	0.037	0.063	—	0.963	0.955
13. LALMF	0.125	0.125	0.085	0.242	0.182	0.158	0.129	0.086	0.128	0.085	0.104	0.038	—	0.948
14. LASAL	0.115	0.126	0.083	0.220	0.213	0.194	0.134	0.072	0.103	0.099	0.053	0.046	0.053	—

Oliva-Tejera et al. (2005), seed dispersal through barochory (i.e., gravity dispersal) in *Lotus* must be a paramount factor to explain the observed heterozygote deficit in most populations because it fosters small genetic neighborhoods where reproduction takes place between related individuals. Furthermore, because our sampling design attempted to represent the distribution of individuals within the population area, the distances between sampled plants in sizeable populations were often large and may possibly have resulted in a Wahlund effect (Wahlund, 1928), thus contributing to the high F_{IS} values. However, only prolonged inbreeding (and not a Wahlund effect) is the most feasible explanation for the high F_{IS} values in *L. kunkelii* and in LASAL and LALMF (the two smallest populations of *L. arinagensis*) because our sampling in these populations represents a high proportion of the visible individuals.

Basic population genetics theory predicts a dramatic loss of genetic variation in small populations due to an enhanced action of drift and inbreeding (Barrett and Kohn, 1991). Therefore, the genetic depauperation of *L. kunkelii* could be construed as a consequence of its demographic traits and of its sharp geographic isolation (it occurs on a secluded slope of a cliff facing the sea at the east of Gran Canaria). Although the bottleneck test was not significant for this population (data not shown), we consider it likely that reductions in population size due to recent anthropogenic disturbances might have affected its levels of variation. Another likely consequence of isolation and low population size in *L. kunkelii* is an increased frequency of inbred matings, as suggested by its value of F_{IS}, the highest of the three taxa examined (Table 2).

In *L. arinagensis* , the sharp differences in population size among LASAL, LALMF, and LAARI (Fig. 1), the fact that the genetic variation of the smallest populations (LALMF and LASAL) is not a strict subset of that in LAARI (Table 1), gravity seed dispersal (that tends to foster reproduction in family clumps) and the homogeneous environmental conditions in its area of distribution hint that the interpopulation genetic homogeneity observed in this taxon is likely a result of recent fragmentation.

By contrast, interpopulation differentiation in the broad-ranging *L. lancerottensis* (Table 3) is more than two-fold that in *L. arinagensis*. At the morphological level, this within-island heterogeneity is illustrated by the population LLJAN from Fuerteventura, that was originally described as *Lotus erythrorhizus* Bolle (Bolle, 1891), later considered by Brand (1898) as *Lotus glaucus* Ait. var. *erythrorhizus* (Bolle) Brand, and finally established as a variety of *Lotus lancerottensis* by Kunkel (1976). At odds with these independent morphological assessments, no substantial difference between LLJAN and the other populations in terms of rare alleles was detected. Although this population has the lowest genetic identity with other populations within Fuerteventura ($I_{NEI} = 0.908$ with LLCOR, Table 4), this value is within the range expected for conspecific populations. In the absence of evidence for local adaptation, the most likely hypothesis to explain within-island differentiation in *L. lancerottensis* is that genetic differentiation among these populations was a by-product of time since genetic isolation and attendant stochastic forces.

Between islands, the two exclusive alleles detected in Fuerteventura and in Lanzarote (Table 1) probably reflect the contribution of mutation to the observed geographic pattern. Drift is another potentially important factor to explain genetic heterogeneity in oceanic islands (Crawford et al., 1987) and, in the hypothesized context of a long divergence time and low levels of gene flow, it probably played a substantial role in the enhancement of inter-island genetic differentiation in *L. lancerottensis*.

The genetic divergence between L. lancerottensis and L. kunkelii—In order to discuss the extent of evolutionary divergence of the endangered *L. kunkelii* and the broad-ranging *L. lancerottensis*, we will first assess if these two taxa fulfill the definition of an "evolutionarily significant unit" (ESU, Waples 1991): a population or group of populations that (1) is reproductively isolated from other con-specific populations, and (2) represents an important component of the evolutionary legacy of the species. Because ESUs are widely equated with conservation units (Moritz, 1994; Karl and Bowen, 1999), this conceptual framework seems especially well suited to substantiate eventual decisions to protect *L. kunkelii*. Only if *L. kunkelii* and *L. lancerottensis* can be considered independent ESUs are we then justified to evaluate if their degree of genetic divergence is enough to recognize *L. kunkelii* at species rank or the same taxon as a subspecies of *L. lancerottensis*.

To test the degree of reproductive isolation between these taxa, it seems adequate to use the values of F_{ST} (Wright, 1951), because this parameter accounts for the proportion of total variation that can be explained by the differentiation among populations and, therefore, reflects the role of gene flow as a force of genetic cohesion. The second part of the definition of ESU is more difficult to assess quantitatively given the diversity of biological or historical traits that can be interpreted as "evolutionary legacy." According to Waples (1995), this term refers to "the genetic variability that is a product of past evolutionary events and that represents the reservoir upon which future evolutionary potential depends." Because rare alleles may confer survival capabilities to populations after an environmental contingency (Schonewald-Cox et al., 1983), they are important components of the future evolutionary potential of populations, and we used them as indicators of evolutionary legacy in these three taxa of *Lotus*.

Population genetics theory predicts that the population groups where F_{ST} values are higher can be considered to be genetically more heterogeneous, presumably as a consequence of the discontinuation of genetic interchange among the constituent populations (Slatkin, 1985, 1987, 1994). By contrast, higher levels of gene flow among populations should be assumed in groups of populations that have lower F_{ST} values (Slatkin, 1985). The quantitative insight offered by this parameter shows that the inclusion of *L. kunkelii* in any artificial assemblage with the other two taxa examined induces a substantial increase in the values of F_{ST} ($F_{ST} = 0.215$ for *L. kunkelii* + *L. lancerottensis*, $F_{ST} = 0.162$ for *L. kunkelii* + *L. arinagensis*, and $F_{ST} = 0.205$ for *L. lancerottensis* + *L. arinagensis*). This evidence indicates that the taxonomic inclusion of *L. kunkelii* within either *L. lancerottensis* or *L. arinagensis* would disrupt the genetic cohesion of these two taxa severely and that population genetic differentiation between *L. lancerottensis* and *L. kunkelii* is even higher than that between *L. lancerottensis* and *L. arinagensis*, whose taxonomic position as different species is undisputed. Qualitatively, although we did not detect substantial "evolutionary legacy" in *L. kunkelii* (only one exclusive allele, Table 1) this taxon shares more alleles with *L. arinagensis* (20 of 21) than with *L. lancerottensis* (15 of 21) (Table 1). These two

genetic results bolster the consideration of *L. kunkelii* and *L. lancerottensis* as independent ESUs according to Waples' (1991) definition.

Most relevant for assessing the extent of genetic divergence between *L. kunkelii* and *L. lancerottensis*, the average genetic identity between these two taxa ($I_{NEI} = 0.823$) is far lower than that detected level for any other pair of Canarian *Lotus* species examined with allozyme electrophoresis ($I_{NEI} = 0.938$ between *L. spartioides* and *L. holosericeus*, Oliva-Tejera et al., 2005), and even lower than the average I_{NEI} between *L. kunkelii* and *L. arinagensis* ($I_{NEI} = 0.886$). Furthermore, the minimum values of I_{NEI} were detected between *L. kunkelii* and some populations of *L. lancerottensis* (Table 4). Although the allozyme genetic evidence compellingly suggests that *L. kunkelii* is much closer to *L. arinagensis* than to *L. lancerottensis* (Fig. 2), it would be incorrect to infer from our results that *L. kunkelii* and *L. arinagensis* should be merged into a single ESU because they are distinct both genetically and morphologically; the rationale of including *L. arinagensis* in these analyses was to provide an external reference to gauge the genetic differences between *L. kunkelii* and *L. lancerottensis*, which were the only source of taxonomic discrepancies. Overall, these comparisons indicate that the range of genetic divergence between *L. kunkelii* and *L. lancerottensis* corresponds to that expected for different species of Canarian *Lotus*.

Conclusion—High genetic variation levels and low interpopulation differentiation in *L. arinagensis* are probably a consequence of a considerable antiquity, habitat stability, and only recent fragmentation. By contrast, *L. lancerottensis* has a substantial interpopulation differentiation, which is best construed as the effect of prolonged action of time since isolation and stochastic forces to increase genetic differentiation both within and between its islands of distribution. *Lotus kunkelii* possesses the lowest levels of variation detected, probably due to increased inbreeding brought about by geographic isolation and by reductions in population size associated with anthropogenic disturbance and recurrent bottlenecks. Overall, our results consistently show that (1) the extent of evolutionary divergence between the endangered *L. kunkelii* and the broad-ranging *L. lancerottensis* is much higher than that detected between either *L. lancerottensis* and the narrowly distributed *L. arinagensis* or between other Canarian *Lotus* examined with allozymes (Oliva-Tejera et al. 2005) and that (2) at odds with Esteve-Chueca's (1972) taxonomic proposal, *L. kunkelii* is genetically closer to *L. arinagensis* than to *L. lancerottensis*. These conclusions agree with the Bramwell and Davis (1972) classification and support the consideration of *L. kunkelii* as a distinct species, whose extremely endangered population should be given maximum protection according to the new methodology for the catalog of threatened Canarian species (Martín-Esquivel 2004).

LITERATURE CITED

BAÑARES, A., G. BLANCA, J. GÜEMES, J. C. MORENO, AND S. ORTIZ [EDS.]. 2003. Atlas y libro rojo de la flora vascular amenazada de España. Dirección General de Conservación de la Naturaleza, Madrid, Spain.

BARRETT, S. C. H., AND J. R. KOHN. 1991. Genetic and evolutionary consequences of small population size in plants: implications for conservation. *In* D. A. Falk and K. E. Holsinger [eds.], Genetics and conservation in rare plants, 3–30. Oxford University Press, New York, New York, USA.

BOLETÍN OFICIAL DE CANARIAS. 1987. Ley 12/1987. no. 85, 1 July. Santa Cruz de Tenerife, Spain.

BOLETÍN OFICIAL DE CANARIAS. 1994. Ley 12/1994. no. 157, 24 December 1994. Santa Cruz de Tenerife, Spain.

BOLLE, C. 1891. Florula insularum olim purpuriarumm, nunc Lanzarote et Fuerteventura cum minoribus isletas de Lobos et Graciosa in Archipielago Canariensis. *Botanische Jahrbücher für Systematik* 14: 230–257.

BRAMWELL, D. 1995. A new *Lotus* species from Gran Canaria. *Botánica Macaronésica* 22: 113–116.

BRAMWELL, D., AND D. D. DAVIS. 1972. A contribution to the study of *Lotus* L. on Gran Canaria. *Cuadernos de Botánica Canaria* 16: 51–54.

BRAND, A. 1898. Monographie der Gattung Lotus. *Botanische Jahrbücher für Systematik* 25: 166–232.

BRITTEN, H. B., AND P. F. BRUSSARD. 1992. Genetic divergence and the Pleistocene history of the alpine butterflies *Boloria improba* (Nymphalidae) and the endangered *Boloria acrocnema* (Nymphalidae) in western North America. *Canadian Journal of Zoology*. 70: 539–548.

CABRITA, L. F., U. AKSOY, S. HEPAKSOY, AND J. M. LEITÃO. 2001. Suitability of isozyme, RAPD and AFLP markers to assess genetic differences and relatedness among fig (*Ficus carica* L.) clones. *Scientific Horticulture* 87: 261–273.

CAUJAPÉ-CASTELLS, J. 2004. Manual para el muestro genético de poblaciones naturales de plantas vasculares. Jardín Botánico Canario "Viera y Clavijo." Cabildo de Gran Canaria, Las Palmas de Gran Canaria, Spain.

CAUJAPÉ-CASTELLS, J., AND M. BACCARANI-ROSAS. 2005. Transformer-3: a program for the analysis of molecular population genetic data. EXEGEN Software and Jardín Botánico Canario "Viera y Clavijo", Las Palmas de Gran Canaria, Spain. Website http://www.exegen.org, http://www.jardincanario.org.

CAUJAPÉ-CASTELLS, J., J. PÉREZ DE PAZ, R. FEBLES, O. FERNÁNDEZ PALACIOS, C. SUÁREZ, J. NAVARRO, M. OLANGUA, R. JAÉN, F. SOSA, AND R. TORRENT. 2001. Manual del laboratorio de DNA del Jardín Botánico Canario "Viera y Clavijo." I. Isoenzimas. Jardín Botánico Canario "Viera y Clavijo." Cabildo de Gran Canaria, Las Palmas de Gran Canaria, Spain.

CORNUET, J. M., AND G. LUIKART. 1996. Description and evaluation of two tests for detecting recent bottlenecks. *Genetics* 144: 2001–2014.

CRAWFORD, D. J., E. RUIZ, T. F. STUESSY, E. TEPE, P. AQEVEQUE, F. GONZALEZ, R. J. JENSEN, G. J. ANDERSON, G. BERNARDELLO, C. M. BAEZA, U. SWENSON, AND O. M. SILVA. 2001. Allozyme diversity in endemic flowering plant species of the Juan Fernandez Archipelago, Chile: ecological and historical factors with implications for conservation. *American Journal of Botany* 88: 2195–2203.

CRAWFORD, D. J., R. WHITKUS, AND T. F. STUESSY. 1987. Plant evolution and speciation on oceanic islands. *In* K. M. Urbanska [ed.], Differentiation patterns in higher plants, 183–199. Academic Press, London, UK.

DEJOODE, D. R., AND J. F. WENDEL. 1992. Genetic diversity and origin of the Hawaiian Island cotton, *Gossypium tomentosum*. *American Journal of Botany* 79: 1311–1319.

DOEBLEY, J., A. STEC, J. WENDEL, AND M. EDWARDS. 1990. Genetic and morphological analysis of a maize-teosinte F2 population: implications for the origin of maize. *Proceedings of the National Academy of Sciences, USA* 87: 9888–9892.

ELISIÁRIO, P. J., E. M. JUSTO, AND J. M. LEITAO. 1999. Identification of mandarin hybrids by isozyme and RAPD analysis. *Scientific Horticulture* 81: 287–299.

ESTEVE-CHUECA, F. 1972. Nuevas referencias a la vegetación litoral de Gran Canaria. *Cuadernos de Botánica Canaria* 14–15: 44.

FRANCISCO-ORTEGA, J., A. SANTOS-GUERRA, S. C. KIM, AND D. J. CRAWFORD. 2000. Plant genetic diversity in the Canary Islands: a conservation perspective. *American Journal of Botany* 87: 909–909.

GAUTHIER, P., R. LUMARET, AND A. BÉDÉCARRATS. 1998. Genetic variation and gene flow in alpine diploid and tetraploid populations of *Lotus* (*L.*

alpinus (D.C.) Schleicher/*L. corniculatus* L.). I. Insights from morphological and allozyme markers. *Heredity* 80: 683–693.

HAMRICK, J. L., AND M. J. W. GODT. 1989. Allozyme diversity in plant species. *In* A. H. D. Brown, M. T. Clegg, A. L. Kahler, and B. S. Weir [eds.], Plant population genetics, breeding and germplasm resources, 43–63. Sinauer, Sunderland, Massachusetts, USA

HARTL, D. L., AND A. G. CLARK. 1989. Principles of population genetics, 2nd ed. Sinauer, Sunderland, Massachusetts, USA.

HELENURM, K. 2001. High levels of genetic polymorphism in the insular endemic herb *Jepsonia malviflora*. *Journal of Heredity* 92: 427–432.

KARL, S. A., AND B. E. BOWEN. 1999. Evolutionarily significant units versus geopolitical taxonomy: molecular systematics of an endangered sea turtle (genus *Chelonia*). *Conservation Biology* 13: 990–999.

KIMURA, M., AND J. F. CROW. 1964. The number of alleles that can be maintained in a finite population. *Genetics* 49: 725–738.

KOORNNEEF, M., C. ALONSO-BLANCO, AND D. VREUGDENHIL. 2004. Naturally occurring genetic variation in *Arabidopsis thaliana*. *Annual Review of Plant Biology* 55: 141–172.

KUNKEL, G. 1976. Cuatro nuevas combinaciones nomenclatóricas para las islas orientales. *Cuadernos de Botánica Canaria* 28: 11–12.

LARSEN, K. 1956. Cyto-taxonomical studies in Lotus III: some new chromosome numbers. *Botanisk Tidsskrift* 53(1): 49–56.

LEWIS, P. O., AND R. WHITKUS. 1993. GeneStat-PC, version 3.3. North Carolina State University, Raleigh, North Carolina, USA.

LUIKART, G., AND J. M. CORNUET. 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conservation Biology* 12: 228–237.

MARTÍN-ESQUIVEL, J. L. 2004. Propuesta metodológica para la catalogación de especies amenazadas en Canarias. *In* J. M. Fernández-Palacios and C. Morici [eds.] Ecología insular/island ecology, 385–412. Asociación Española de Ecología Terrestre (AEET)-Cabildo Insular de La Palma, Santa Cruz de La Palma, Spain.

MORITZ, C. 1994. Defining 'evolutionarily significant units' for conservation. *Trends in Ecology and Evolution* 9: 373–375.

NEI, M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences, USA* 70: 3321–3323.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583–590.

OLIVA-TEJERA, F., J. CAUJAPÉ-CASTELLS, J. NARANJO-SUÁREZ, J. NAVARRO-DÉNIZ, J. R. ACEBES-GINOVÉS, AND D. BRAMWELL. 2005. Population genetic differentiation in taxa of *Lotus* (Fabaceae: Loteae) endemic to the Gran Canarian pine forest. *Heredity* 94: 199–206.

ORTEGA, J. 1976. Citogenética del género *Lotus* en Macaronesia. *Botánica Macaronésica* 1: 17–24.

PIRY, S., G. LUIKART, AND J. M. CORNUET. 1998. Bottleneck, a program for detecting recent effective population size reductions from allele frequency data. Laboratoire de Modélisation et Biologie Evolutive, Institut National de la Recherche Agronomique, Montpellier, France.

REED, D. H., AND R. FRANKHAM. 2001. How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. *Evolution* 55: 1095–1103.

ROGERS, J. S. 1972. Measures of genetic similarity and genetic distance. *Studies in Genetics, University of Texas Publication* 7213: 145–153.

ROGERS, J. S. 1991. A comparison of the suitability of the Rogers, modified Rogers, Manhattan, Cavalli-Sforza and Edwards distances for inferring phylogenetic trees from allele frequencies. *Systematic Zoology* 40: 63–73.

SCHONEWALD-COX, C. M., S. M. CHAMBERS, B. MACBRIDE, AND W. L. THOMAS [EDS.]. 1983. Genetics and conservation: a reference for managing wild animal and plant populations. Benjamin Cummings, Menlo Park, California, USA.

SLATKIN, M. 1985. Gene flow in natural populations. *Annual Review of Ecology and Systematics* 16: 393–430.

SLATKIN, M. 1987. Gene flow and the geographic structure of natural populations. *Science* 236: 787–792.

SLATKIN, M. 1994. Gene flow and population structure. *In* L. A. Real [ed.], Ecological genetics, 3–17. Princeton University Press, Princeton, New Jersey, USA.

SWOFFORD, D. L., AND R. B. SELANDER. 1989. BYOSYS-1: a fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *Journal of Heredity* 72: 281–283.

WAHLUND, S. 1928. Zusammensetzung von populationen und korreletionsercheinungen vom standpunkt der vererbungslehre aus betrachtet. *Hereditas* 11: 65–105.

WAPLES, R. 1991. Genetic interactions between hatchery and wild salmonids: lessons from the Pacific Northwest. *Canadian Journal of Fisheries and Aquatic Sciences* 48: 124–133.

WAPLES, R. 1995. Evolutionarily significant units and the conservation of biological diversity under the Endangered Species Act. *American Fisheries Society Symposium* 17: 8–27.

WATTERSON, G. A. 1978. The homozygosity test of neutrality. *Genetics* 88: 405–417.

WRIGHT, S. 1951. The genetical structure of populations. *Annals of Eugenics* 15: 323–354.

YEH, F. C., R. C. YANG, T. B. J. BOYLE, Z. H. YE, AND J. X. MAO. 1997. PopGene version 1.32, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada.