



Phylogeographic analysis points toward invasion of the Timanfaya National Park (Lanzarote; Canary Islands) by a translocated native plant (*Rumex lunaria*)

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Abstract

Rumex lunaria is an endemic shrub of the Canary Islands, which is colonizing the Timanfaya National Park (TNP) in Lanzarote. Whether the arrival of *R. lunaria* to Lanzarote has been natural or by human intervention is still a matter of debate. To address this question, 100 specimens of *R. lunaria* were collected from the seven main Canary Islands, and genetic analysis of four chloroplast DNA *loci* were performed, covering a total length of 4809 nucleotide positions. Multiple alignments revealed 49 nucleotide substitutions, which define 30 different haplotypes. Island-specific haplotypes were found in Tenerife, La Gomera, La Palma and Gran Canaria, with the greatest diversity found in the first island. Interestingly, the unique haplotype detected in El Hierro is shared with almost all plants from Lanzarote (95%), including all individuals sampled in the TNP. The most frequent haplotype present in Gran Canaria was detected in only one sample from Lanzarote (5%). These results were corroborated by a robust phylogenetic analysis, which supports the hypothesis of a common origin of *R. lunaria* populations from El Hierro and the vast majority of those from Lanzarote. In addition, this study rules out the genetic singularity of the *R. lunaria* specimens that are colonizing the TNP.

Keywords Canary Islands · Chloroplast markers · Plant endemism · *Rumex lunaria* · Translocated native species

Introduction

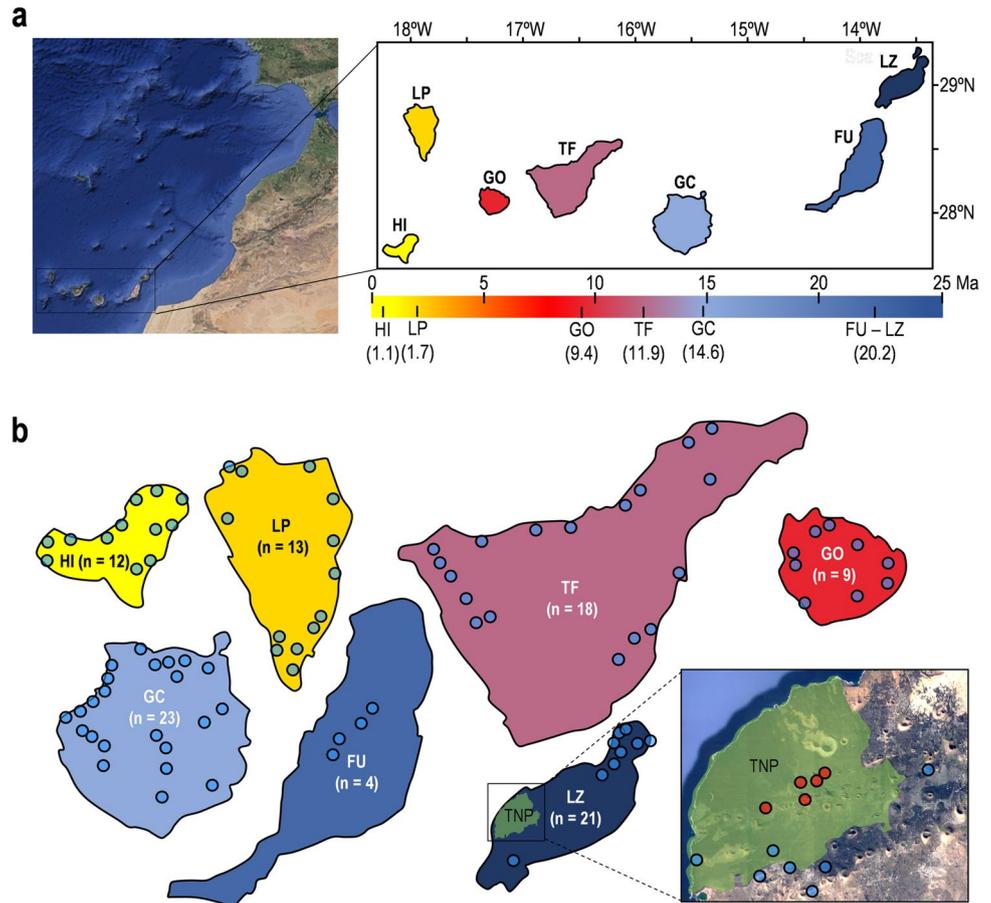
Canary Islands represent an excellent model for phylogeographic analysis because, as in other oceanic archipelagos, the geographical barriers of island-specific populations are clearly defined (Crawford and Stuessy 1997). The Canary Islands have a west–east age gradient, with El Hierro (HI) as the youngest island, followed by La Palma (LP), La Gomera (GO), Tenerife (TF), Gran Canaria (GC), and Fuerteventura

(FU) - Lanzarote (LZ) (Anguita and Hernán 2000; Carracedo and Troll 2013) (Fig. 1a). The Canary archipelago belongs to the “Mediterranean basin”, one of the most remarkable biodiversity hotspots worldwide (Myers et al. 2000), and contains more than 570 endemic plant species (about 40% of all plant species described in the islands). Some of these endemic species are distributed throughout the whole archipelago, while others are restricted to certain islands (Francisco-Ortega et al. 2000). The spread of species between islands may be influenced by several factors, such as dispersal vectors, number of introduced individuals, adaptation to a new environment and biotic interactions with native species (Bruno et al. 2005; Jones and Gomulkiewicz 2012). Human activity is transferring island-endemic species from one island to another, causing environmental issues, which are even more serious in fragile island ecosystems. Therefore, to assess whether an island-specific endemism has been naturally dispersed or transported by human action to other islands, represents a challenge for conservation biology in this region. In this scenario, analysis of genetic variability of invasive plant species represents a valuable tool,

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Fig. 1 Sampling of *Rumex lunaria* populations in Canary Islands and PCR amplification of cpDNA loci. **a** Geographical location of the Canary archipelago and estimated geological ages for each island, which are highlighted with a color code. **b** Number (n) and location of *R. lunaria* individuals analyzed in this study, including five individuals sampled inside the TNP (LZ). Ma: Millions of years; HI: El Hierro; LP: La Palma; TF: Tenerife; GO: La Gomera; >GC: Gran Canaria; FU: Fuerteventura; LZ: Lanzarote; TNP: Timanfaya National Park.



since results can be used to estimate the human influence over the species distribution, allowing to achieve the best decision for the species management (Kardos 2021).

Rumex lunaria L., is an endemic shrubby plant from Canary Islands. It has been described as a polygamous species with a gynodioecious reproductive system, considered an intermediate evolutionary step between monoicy and dioicy (Löve 1943; Navajas-Pérez et al. 2005). The genus *Rumex* belongs to *Polygonaceae* family, in which the species *R. lunaria* is included in the subgenus *Acetosa* (section *Hastati*). This species has been cited among the rare edible plant species of the Canary Islands (Darias et al. 1993), and was found to produce phytochemical compounds of medical interest (Rodríguez de Vera et al. 2004, 2007; Tonny et al. 2017; Mishra et al. 2018; Froidi et al. 2020). *R. lunaria* has been traditionally used as forage, mainly as a consequence of its good adaptation to arid environments (Ventura et al. 2004; Alvarez et al. 2008; Arévalo et al. 2012).

Currently, *R. lunaria* is widespread distributed in western islands (HI, LP, GO, and TF), as well as in GC. Oral sources

mention the transport of *R. lunaria* seeds from El Hierro to Lanzarote for crop, in the early 1900s. People emphasize that the common name of “calcosa”, by which this species was known at that time in Lanzarote, is used by people from El Hierro only, while in the rest of the islands it was called, and is still called, “vinagrera” (López 2006). The first known reference to the presence of *Rumex lunaria* on LZ comes from the Norwegian botanist Per Sunding (Sunding 1970). Shortly after, it was considered a putative invasive species in LZ, because new plant individuals were detected in 1987 near the Timanfaya National Park (TNP) (Wildpret et al. 1995; Betancort 1999). Due to its ability to colonize volcanic territories, especially pyroclastic areas, *R. lunaria* is nowadays transforming the singular and fragile landscape of the TNP. However, objective evidences of human intervention are needed before considering the expansion of *R. lunaria* in LZ as an invasive process, before setting up control and management actions. Faced with this situation, we carried out a genetic study aimed to trace the origin of *R. lunaria* populations in LZ, and to determine if these populations show any genetic peculiarity that is worthy of protection.

Methods

Sample collection and DNA purification

A total of 100 *R. lunaria* individuals were sampled from the seven main Canary Islands. The area of each island, and the relative abundance of *R. lunaria* populations (Gobierno de Canarias 2022) were considered to collect a representative number of individuals. The low number of individuals sampled in FU reflects the extremely low abundance of *R. Lunaria* specimens. In fact, the four samples from FU were collected in urban gardens or in farmland next to houses. One individual from TF belonging to the closely related species *R. vesicarius* was included as outgroup for the indicated genetic analysis. GPS coordinates and additional information on each sample can be found in Supplementary Information (Table S1).

DNA was extracted from 30 mg of silica gel-dried leaf tissue and homogenized in 2 ml Lysing Matrix-A tubes (M.P. Bio-medicals, USA) by vigorous shaking (5 m/s; 30 s), in a FastPrep-24 system (M.P. Biomedicals, USA). An initial homogenization cycle without adding lysis buffer was included, and genomic DNA was purified from the lysate using the E.Z.N.A DS Plant DNA Kit (Omega Bio-Tek, USA), following manufacturer's recommendations. DNA was recovered in 50 µl of elution buffer supplied with the kit, and DNA concentration and purity were determined with a DeNovix DS-11 spectrophotometer (Denovix, USA). Each DNA sample was diluted to a final concentration of 10 ng/µl using 10 mM Tris-HCl, pH 8.0, and stored at – 20 °C. For PCR amplification dilutions were prepared at 0.4 ng/µl in the same buffer.

PCR amplification and DNA sequencing

Four chloroplast DNA (cpDNA) *loci* were selected for this study. Oligonucleotides, used as primers for PCR amplification and/or for amplicon sequencing, were taken from the existing literature or designed specifically for this work (Table 1). The first amplicon includes the spacer region between genes encoding leucine-tRNA for UAA codon (*trnL-UAA*) and threonine-tRNA for UGU codon (*trnT-UGU*). The second amplicon includes the intron of lysine-tRNA gene for UUU codon (*trnK-UUU*), as well as the coding region of the maturase protein (*matK*). The third amplicon encompasses the DNA fragment placed between genes encoding aspartic-tRNA for GUC codon (*trnD-GUC*) and threonine-tRNA for GGU codon (*trnT-GGU*), including three intergenic sequences and two tRNA genes (*trnE-UUC* and *trnY-GUA*). The last fragment includes the cpDNA region between cysteine-tRNA gene for GCA codon (*trnC-GCA*), and the gene encoding B subunit of RNA polymerase (*rpoB*).

PCR reactions contained 2 ng of template genomic DNA, 1X Phire Hot Start II Reaction Buffer (Thermo Fisher Scientific, USA), 0.2 mM each dNTP (VWR, USA), 0.2 µM of each amplification primer, 0.2 µl Phire Hot Start II DNA polymerase (Thermo Fisher Scientific, USA), and H₂O to obtain a final volume of 20 µl. A ProFlex PCR System (Applied Biosystems, USA) was used for incubation of PCR reactions with the following thermal cycling conditions: initial denaturation step at 98 °C for 30 s; 35 amplification cycles with denaturation at 98 °C for 10 s, annealing at the temperature indicated in Table 1 for 10 s; extension at 72 °C during the time indicated in Table 1; and a final extension

Table 1 Oligonucleotides used in the present study and PCR conditions

Chloroplast DNA <i>loci</i>	Primer sequence (5'–3')	Annealing temperature (°C)	Extension time (s)	Amplicon length (Kb)
<i>trnL-UAA/trnT-UGU</i>	TCTACCGATTTTCGCCATATC ^{a, c} CATTACAAATGCGATGCTCT ^{a, c} CTTTCATTTCTAGTGGGAGC ^{b, e}	54.0	15	0.8
<i>trnK-UUU(matK)</i>	GGGTTGCCCGGGACTCGAAC ^{a, d} CAACGGTAGAGTACTCGGCTTTTA ^{a, d} CATCGGTAGAGTTTGTAAGAC ^{b, e} GCTTTCCTATGTACACATCT ^{b, e}	63.0	30	2.5
<i>trnD-GUC/trnT-GGU</i>	ACCAATTGAACTACAATCCC ^{a, d} CTACCACTGAGTTAAARGGG ^{a, b, d} CAGGAAATAAGGTATAACG ^{b, e}	53.0	20	1.5
<i>trnC-GCA/rpoB</i>	GGCGACACCCAGATTTGAAC ^{a, e} TCCACCAATTGATATGTTTCCAC ^{a, e} GCAGTCCTCCGCTTAC ^{b, e}	61.0	15	1.1

^aUsed for PCR amplification. ^bUsed for Sanger sequencing. ^cDesigned by (Taberlet et al. 1991). ^dDesigned by (Demesure et al. 1995). ^eDesigned in the present work

step at the same temperature and time that previous amplification cycles.

Before DNA sequencing, PCR products were checked by gel electrophoresis in 2% agarose gels prepared in 1X TBE buffer. PCR products were enzymatically purified with the ExoCleanUp reagent (VWR, USA), and sequenced by the Sanger method with the primers indicated in Table 1, making use of an external service (Macrogen Inc). Sequencing electropherograms were manually inspected with the MEGA X software (Kumar et al. 2018), in order to discard low quality reads and to trim primer sequences when present.

DNA sequence alignment and data analysis

Sequences obtained for each cpDNA locus were separately aligned with the ClustalW algorithm (Thompson et al. 1994; Larkin et al. 2007) included in the MEGA X software (Kumar et al. 2018). Alignments were manually inspected to confirm gap positions and, when necessary, were trimmed from both ends to generate a rectangular-matrix dataset. A concatenated alignment was then obtained using DnaSP software 6.0 (Rozas et al. 2017) by putting together sequences from the four cpDNA *loci*.

The number of polymorphic (segregating) sites (S), number of haplotypes (H), haplotype diversity (Hd), and nucleotide diversity (π) were calculated with DnaSP 6.0 (Rozas et al. 2017). Relationships among the haplotypes were depicted with median-joining (MJ) networks (Bandelt et al. 1999) using PopART v1.7 (Leigh and Bryant 2015), excluding indel mutations and setting Epsilon to zero. Pairwise F_{ST} values among islands, as well as AMOVA analysis, were carried out with Arlequin v3.5.2.2 software (Excoffier et al. 2007).

Phylogenetic inferences were carried out at the CIPRES portal (Miller et al. 2010), using the concatenated alignment. F81 was selected as the best substitution model based on the Bayesian Information Criterion (BIC) and Akaike information criteria (AIC), calculated separately for each *loci*, using both ModelFinder (Kalyaanamoorthy et al. 2017) implemented on IQ-TREE 2 tool (Minh et al. 2020). Maximum Likelihood (ML) phylogeny was generated using RaxML v8.2.12 (Stamatakis 2014), including 1000 bootstrap replicates, and unlinked the four cpDNA *loci*. Additionally, Bayesian phylogenetic tree was reconstructed using MrBayes v3.2.7 (Ronquist et al. 2012), using two independent runs of four calculation chains, ran simultaneously for 10^7 generations each, and sampling every 100 generations. The first 2.5×10^4 strings were discarded as burn-in, and the four cpDNA *loci* were unlinked during calculations. The consensus tree was then visualized and edited with FigTree

software v1.4.4 (Rambaut 2018). Orthologous sequences of *R. vesicarius* were used as outgroups.

Results

Phylogeographic patterns of cpDNA variation in *R. lunaria* populations

One hundred individuals of *R. lunaria*, which represent the main populations of this species in the Canary Islands (Gobierno de Canarias 2022), were sampled in the present study. Among them, five individuals from the TNP (LZ) were included (Fig. 1b). Optimized PCR conditions allowed us to obtain the expected amplicon for each cpDNA *loci* marker (Table 1), without contamination or unspecific amplification products. High-quality sequences for all samples were obtained and deposited at the NCBI GenBank (Table S1).

The alignment of concatenated DNA sequences from the four cpDNA *loci*, and the 100 *R. lunaria* individuals, resulted in 4,809 aligned sites. Among them, 50 positions were affected by gaps of different length, which were excluded from subsequent analysis. In the final dataset (4759 sites) a total of 49 positions were variable, being 33 of them parsimony-informative and 16 singleton variants (Table S2). FU and LP showed the highest nucleotide diversity while HI and LZ the lowest, being the overall nucleotide diversity $1.54 \times 10^{-3} \pm 7.0 \times 10^{-5}$ (Table 2). Considering the 49 nucleotide substitutions, a total of 30 cpDNA haplotypes were detected (Table S2), which led to a high haplotype diversity when populations from all seven islands were considered together (0.859 ± 0.029) (Table 2). TF and LP showed the highest haplotype diversity while HI and LZ, again, the lowest. It is noteworthy that both nucleotide and haplotype diversity were zero in HI because only one cpDNA haplotype was detected in this island, whereas the high values for these genetic diversity parameters encountered in FU are due to the fact that 2 highly divergent haplotypes were detected with only four sampled individuals.

Interestingly, between 4 and 11 island-specific haplotypes were found in LP, GO, TF and GC islands, whereas the unique haplotype present in HI (H1) was shared with LZ and FU. Additionally, in these last two islands was detected the haplotype most frequently found in GC (H25; Fig. 2a; Table S2). Intriguingly, while the presence of a single haplotype is consistent with the relatively young geological origin of HI island, the presence of only 2 haplotypes is discordant with the longer evolutionary time in the cases of LZ and FU (Fig. 1a).

Table 2 cpDNA genetic diversity detected in *R. lunaria* populations

Population (Island) ^a	No. of samples	No. of sites ^c	No. of polymorphic sites (S)	No. and name (H) of haplotypes	Haplotype diversity (Hd ± SD)	Nucleotide diversity (π ± SD)
HI	12	4772	0	1 (H1)	0.0 ± 0.0	0.0 ± 0.0
LP	13	4771	11	6 (H2-H7)	0.821 ± 0.082	$7.10 \times 10^{-4} \pm 1.5 \times 10^{-4}$
GO	9	4791	5	4 (H8-H11)	0.750 ± 0.112	$4.30 \times 10^{-4} \pm 5.0 \times 10^{-5}$
TF	18	4769	13	11 (H12-H22)	0.928 ± 0.04	$5.60 \times 10^{-4} \pm 7.0 \times 10^{-5}$
GC	23	4776	8	8 (H23-H30)	0.739 ± 0.081	$3.80 \times 10^{-4} \pm 6.0 \times 10^{-5}$
LZ	21	4772	11	2 (H1, H25)	0.095 ± 0.084	$2.20 \times 10^{-4} \pm 1.9 \times 10^{-4}$
FU	4 ^b	4772	11	2 (H1, H25)	0.667 ± 0.204	$1.54 \times 10^{-3} \pm 4.7 \times 10^{-4}$
TOTAL	100	4759	49	30	0.859 ± 0.029	$1.54 \times 10^{-3} \pm 7.0 \times 10^{-5}$

^aHI: El Hierro; LP: La Palma; TF: Tenerife; GO: La Gomera; GC: Gran Canaria; FU: Fuerteventura, LZ: Lanzarote;

^bThe low sample number in FU reflects the scarcity of natural *R. lunaria* specimens in this island

^cExcluding gaps

Evolutionary relationships among haplotypes were inferred by construction of a median-joining haplotype network (Fig. 2a), which again evidences the high haplotype diversity in LP, GO, TF, and GC, and the absence of island-exclusive haplotypes in HI, LZ and FU. The most basal divergence event seems to have occurred between GC and the most western islands (TF, GO, LP and HI), which segregates with at least seven mutational events. The next inter-island divergence process appears to have generated the cpDNA lineages of TF, GO and LP/HI. More recently, the cpDNA lineages of LP and HI separated from a common ancestor. The cpDNA haplotypes of *R. Lunaria* has experienced an intra-island diversification in the populations of GC, TF, GO and LP. Moreover, in TF and LP 2 clearly separated cpDNA lineages are currently found. This genetic analysis suggests that haplotype H1, present in HI, LZ and FU (100, 95 and 50% of samples, respectively) had a unique origin, as well as haplotype H25 detected in GC, LZ and FU (48, 5 and 50% of samples, respectively). Finally, 5 clear haplogroups were detected (Fig. 2a), being haplogroups HG2, HG3 and HG4 island-exclusive (LP, GO and TF islands, respectively), while certain members of haplogroups HG1 and HG5 are present in several islands, as mentioned before (haplotypes H1 and H25). Therefore, these results reveal a clear genetic structure of *R. lunaria* populations.

The pairwise F_{ST} estimations support the interpretation of the haplotype network given above (Fig. 2b). A clear differentiation was detected between GC and the western islands ($F_{ST} = 0.76$ – 0.9), but also between GC and LZ ($F_{ST} = 0.87$), while populations from HI and LZ showed no differentiation ($F_{ST} = 0$). To test this hypothesis, AMOVA analysis were conducted under two possible models, excluding FU in both cases due to the low number of individuals sample in this island. The first model groups LZ and GC islands as one region, while

the second considered LZ in the same region than western islands (Fig. 2c). In the first model, 75.8% of genetic variation was found within regions (i.e. between islands inside each region), while in the second model the differences inside each region decreases to 38.5%, being the differences between regions the most important source of genetic variation. Thus, AMOVA results confirms the hypothesis that *R. lunaria* population from LZ is more genetically related to those from the geographically distant western islands than to the population of the neighboring GC.

Phylogenetic inferences

Topologies of the phylogenetic trees obtained by ML and Bayesian approaches were identical (Fig. 3), revealing the existence of six clades statistically well-supported (Bayesian posterior probabilities between 0.97 and 1). The strong differentiation between *R. lunaria* population from GC (clade-6) and the populations from western islands was corroborated, since HI, LP, GO and TF appear to share a common ancestor. Clade-1 accommodates all individuals from HI, while clades-2 and – 5 gather all the *R. lunaria* specimens from LP and GO, respectively. Individuals from TF were distributed in the clades-3 and – 4, largely in line with their geographical location (south or north, respectively). These clear phylogeographic signals were notably disrupted by samples from FU and LZ, since individuals from these neighbor islands were assigned to 2 phylogenetically distant clades. In the case of LZ, 20 out of 21 samples (including the 5 from TNP) were grouped in clade-1 together with those from HI, while the sample LZ-3, which was collected in an urban area near the locality of Femés, was assigned to clade-6 along with samples from GC. Similarly, *R. lunaria* individuals from FU were half distributed between clade-1 and – 6.

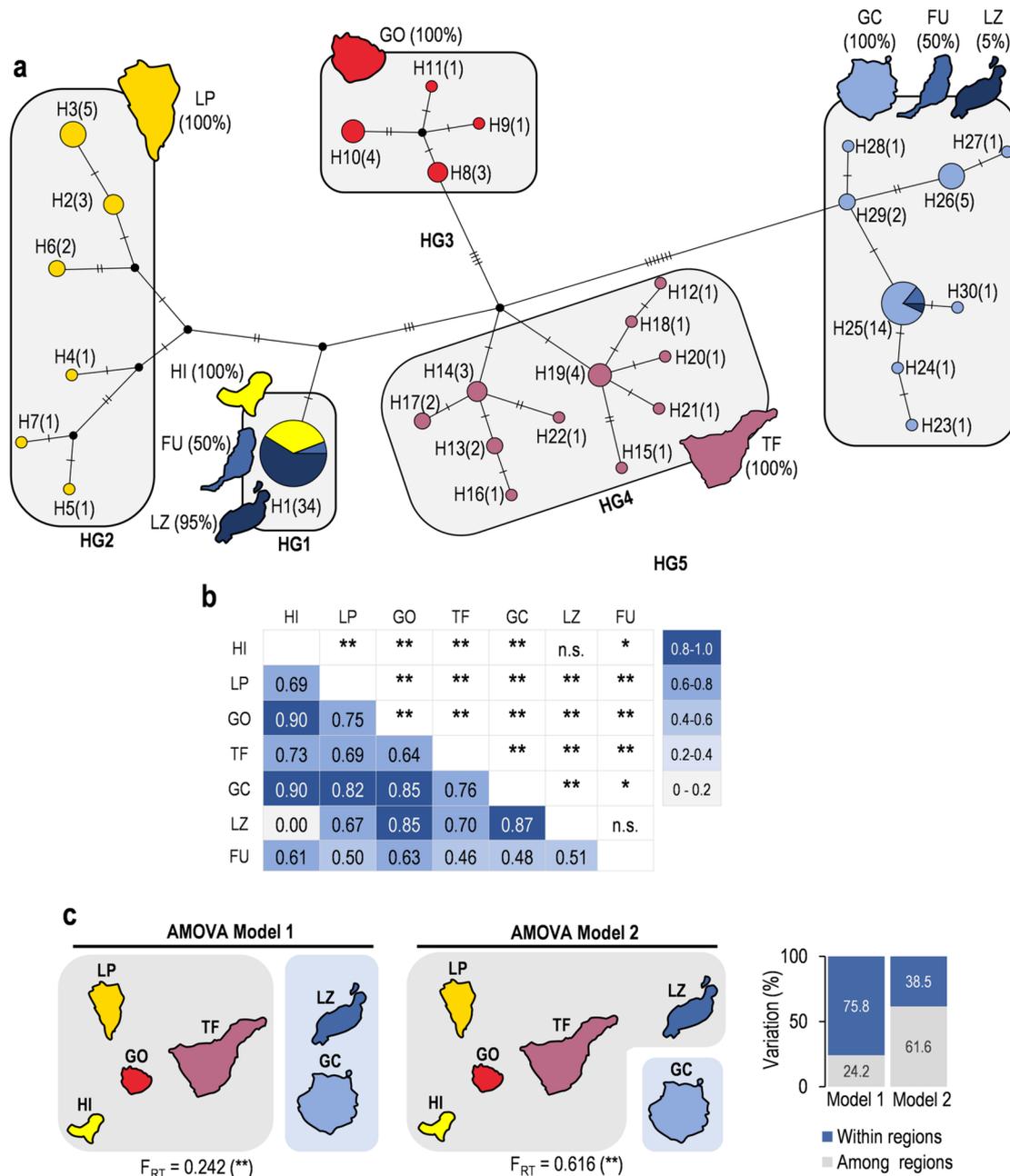


Fig. 2 Phylogeographic analysis of *R. lunaria* populations in the Canary Islands. **a** Median Joining haplotype network for *R. lunaria* cpDNA. The most probable connection is shown for the 30 different haplotypes detected (Table S2). Circle sizes are proportional to the number of individuals with each haplotype (in brackets). Haplogroups (HG1-5) are highlighted in grey, and the percentage of individuals from each island within each haplogroup is shown in brackets. Colors represent the geological age of each island as indicated in Fig. 1a. The mutational events necessary to generate a haplotype from its predecessor are shown with crossed lines, and the most prob-

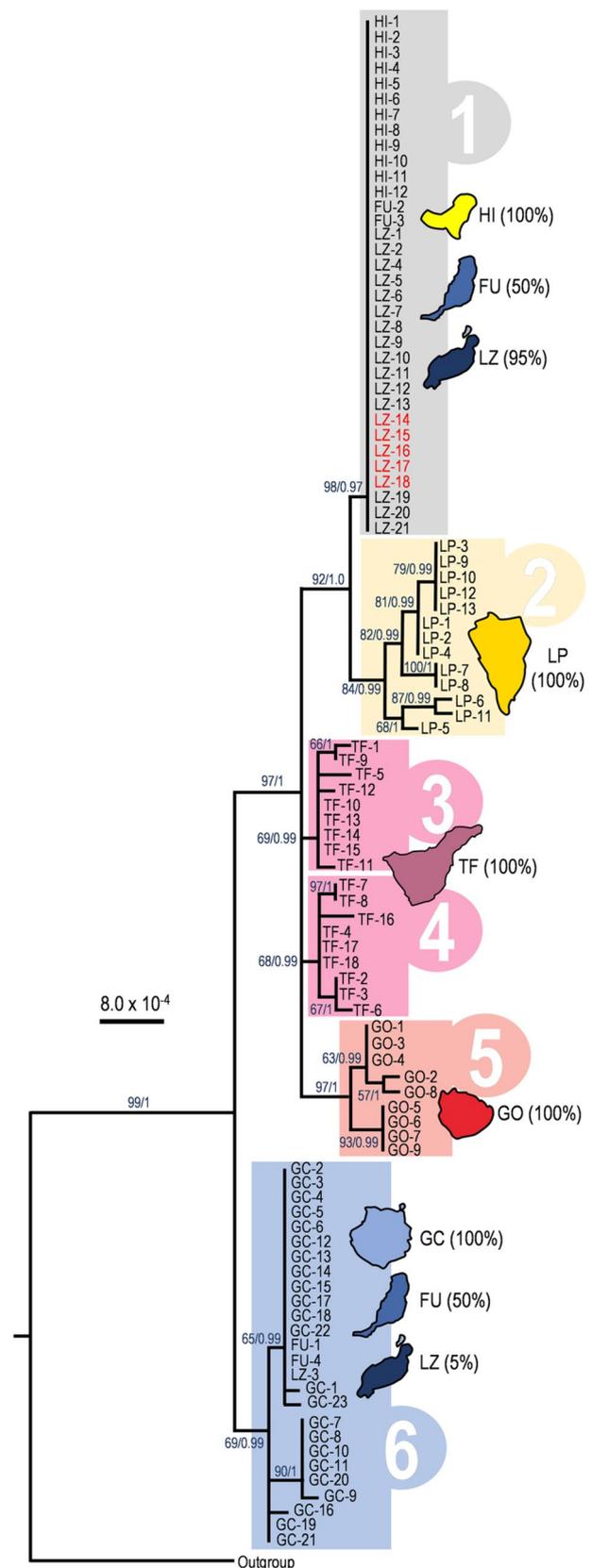
able ancestral haplotypes are shown with black circles. **b** Pairwise F_{ST} values (below diagonal) and statistical significance values (above diagonal), obtained for each possible island combination. **c** Models proposed for AMOVA analysis. Total F_{RT} values are shown at the bottom of each model, and their statistical significance between brackets. Percentage of variation within regions (blue) and among regions (grey) are shown at the left. HI: El Hierro; LP: La Palma; TF: Tenerife; GO: La Gomera; GC: Gran Canaria; FU: Fuerteventura; LZ: Lanzarote; ** $p < 0.01$; * $p < 0.05$; n.s.: not significant

Fig. 3 Phylogenetic relationships detected among the 100 *R. Lunaria* individuals included in the study. The consensus tree obtained by Bayesian inference is displayed. Values over the branches are ML bootstrap percentages and Bayesian posterior probabilities, which indicate the statistical robustness of the tree nodes. Numbers inside colored circles identify the clades referred in the text. The percentage of individuals from each island belonging to a particular clade is shown in brackets. The geological age of each island is indicated by the same color code as in Fig. 1a. Individuals from Timanfaya National Park are highlighted in red. *R. vesicarius* sequences were included as outgroup. Branch lengths reflect genetic distances as indicated by the scale. HI: El Hierro; LP: La Palma; TF: Tenerife; GO: La Gomera; GC: Gran Canaria; FU: Fuerteventura; LZ: Lanzarote

Discussion

R. lunaria is causing important concerns in LZ island due to the recent colonization of the TNP volcanic landscapes (Wildpret et al. 1995; Betancort 1999). Local oral sources indicate that, in the early 1900's, *R. lunaria* was deliberately introduced in Máguez, a rural region in the northeast of LZ, to be used as fodder for goat farming (López 2006; Gil et al. 2009). These sources pointed to HI as the origin of seeds or specimens, citing as evidence the fact that the same common name (*calcosa*) is used in HI and LZ, while this plant species is known under a different popular name (*vinagrera*) in the other islands. In addition, we were unable to find any descriptive work that mentions the presence of *R. lunaria* in LZ prior to that date, which is in consonance with this hypothesis. However, alternative explanations should be considered, as the presence in the island of old seeds with high resilience that have germinated recently (Pérez-García et al. 2008), or long-distance transportation of the winged seeds by a natural vector (Nogales et al. 2012). In the present study, a genetic analysis of *R. lunaria* populations in the Canary Islands has been performed, searching for evidence that either support a natural or human-modified distribution in the Canary Islands, and in order to advise local government on making the right choices for management of this species.

In our study, the highest number of different cpDNA haplotypes was observed in TF, followed by GC, LP and GO. In contrast, in the most western island (HI) we found a single haplotype, which is consistent with being the youngest island of the Canary Archipelago. Paradoxically, despite they are the geologically oldest islands, only 2 different haplotypes were detected in LZ and FU. All haplotypes present in TF, GC, LP and GO, with only one exception in GC, are island specific. However, this peculiarity was not observed in LZ and FU. The biogeographical signal we observed for *R. lunaria* in most of the Canary Islands fits with the predominant pattern described for biological species in this archipelago, which is the result of a stepwise colonization from the older islands in the east, to the younger islands



in the west (Juan et al., 2000). This principle has been designed as the “island progression rule”, in which older land masses donate migrants to younger islands (Whittaker et al. 2017). Indeed, this distribution has been established for many taxa, not only in the Canary Islands, but in different archipelagos (Carvalho et al. 2015). Therefore, species dispersion in volcanic archipelagos usually occurs as a directional process, from older to younger islands, and this distribution has been associated with lower genetic diversity in younger island populations than in older ones. Examples of this distribution pattern can be found in the *R. lunaria* related species *R. bucephalocepharus* (Talavera et al., 2013), but also in distant species, such as *Olea europaea* (García-Verdugo et al. 2009).

Both haplotype network and phylogenetic analysis support a common origin for the vast majority (95%) of the *R. lunaria* individuals sampled in LZ (including all specimens from the TNP), 50% of the individuals from FU and all individuals from HI. Only one sample from LZ, as well as the other 50% from FU, share evolutionary ancestry with the most common haplotype from GC. These observations indicate that evolutionary time has not been enough for the diversification of original haplotypes in LZ and FU. This inconsistency between geological time and haplotype diversity, suggests that *R. lunaria* must have colonized LZ and FU at a much more recent time in the evolutionary scale, thus in line with the oral history in the case of LZ.

Anemochory is the unique natural mechanism of seed dispersal described for *R. lunaria* (Barquin and Wildpret 1975). The lack of haplotypes shared among TF, GC, LP and GO, suggests that natural dispersal of *R. lunaria* between islands is an extremely rare event, even between islands as close as TF and GO which are separated by about 30 km. Further evidence of its limited dispersal capacity is the genetic differentiation of *R. lunaria* populations observed in TF. Moreover, it is highly improbable that a natural migration has occurred between the two most distant islands of the archipelago with no signs of having been in intermediate islands, contrary to the typical case that has been previously observed for other *Rumex* species (Talavera et al. 2013).

Therefore, the migration of *R. lunaria* between HI, LZ and FU has probably happened through human intervention. In this regard, the fact that the most frequent cpDNA haplotype found in LZ is evolutionarily more related to those found in the western islands than to haplotypes from geographically closer islands, is in opposition to the theory that natural and long-distance dispersal increases genetic dissimilarity (Wright 1943). Considering together the distances observed in the haplotype network, phylogenetic relationships and AMOVA results, the most plausible scenario is the migration

of *R. lunaria* from HI to LZ, and from HI or LZ to FU. Overall, if genetic analyses performed in this study were considered, there is no evidence that supports the existence of a genetic singularity that is worthy of protection in the *R. lunaria* population from LZ.

Although initially it was not an objective of the study, our data reveal a fingerprint of human intervention in the distribution of *R. lunaria* in FU. The few plants from this island that could be incorporated to our analyses were collected in highly anthropized areas. Specimens of *R. lunaria* are much less abundant in FU than in LZ. Since FU has not experienced historical or sub-recent volcanic processes, and *R. lunaria* is a pioneer colonizer of this type of areas (Wildpret et al. 1995), a rapid expansion of *R. lunaria* in LZ (worryingly in its national park) might have been favored in comparison to FU. This would be an example of how different ecological factors can drive local adaptation in biological invasions (Rosenthal et al. 2021).

It is well known that human activities exert an important effect over biogeography of island species (Helmus et al. 2014; Graham et al. 2017; Hofman and Rick 2017). In the Canary Islands, translocation of endemic plants with ornamental purposes is a generalized practice. One clear example is the dragon tree (*Dracaena* spp.), which has been cultivated in public or private gardens, and also along main roads between different urban centers, resulting in a non-random distribution of specific haplotypes (Durán et al. 2020). Recently, it has been reported that traffic along roads near the Timanfaya National Park, is modifying distribution of native and non-native species, including *R. lunaria* (Bernardos et al. 2023).

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10592-023-01592-5>.

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Author contributions All authors contributed to the study conception and design. Sample collection was carried out by RC and MBH. Laboratory procedures and data collection were performed by MGC and JPP. Data analysis was carried out by MGC, MHF and JPP. The first draft of the manuscript was written by MGC. All authors read and edited the initial version of the manuscript and approved the final version.

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Data availability All data generated and analyzed in this study are included in the published version of the article and its supplementary information files. Sequences have been deposited at NCBI GenBank (Acc. Numbers can be found at Table S2).

Declarations

Conflict of interest The authors declare no competing interests.

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