

Phylogeography and seed dispersal in islands: the case of *Rumex bucephalophorus* subsp. *canariensis* (Polygonaceae)

María Talavera¹*, Laura Navarro-Sampedro², Pedro L. Ortiz¹ and Montserrat Arista¹

¹Departamento de Biología Vegetal y Ecología, Universidad de Sevilla, Apdo 1095, 41080 Sevilla, Spain and

²Servicio de Biología, Universidad de Sevilla, (CITIUS) Avda. Reina Mercedes 4B, Sevilla, Spain

*For correspondence. E-mail mtalavera@us.es

Received: 3 July 2012 Returned for revision: 25 September 2012 Accepted: 6 November 2012 Published electronically: 23 December 2012

- **Background and Aims** *Rumex bucephalophorus* subsp. *canariensis* is an endemic taxon to Macaronesia with diaspore polymorphism. The origin and colonizing route of this taxon in Macaronesia was studied using molecular data and information on diaspore types.
- **Methods** Amplified fragment length polymorphism (AFLP) was used in 260 plants from 22 populations of *R. bucephalophorus* subsp. *canariensis*, four from the Madeiran archipelago and 18 from the Canary archipelago. Diaspore production was analysed in 9–50 plants from each population used for AFLP analysis. One hundred and one plants from the Madeiran archipelago and 375 plants from the Canary Islands were studied. For each plant the type of diaspore produced was recorded.
- **Key Results** Overall populations had low genetic diversity but they showed a geographical pattern of genetic diversity that was higher in the older eastern islands than in the younger western ones. Two types of dispersible diaspores were found: in the eastern Canary islands (Lanzarote, Fuerteventura and Gran Canaria), plants produced exclusively long-dispersible diaspores, whereas in the western Canary islands (Tenerife, La Gomera, El Hierro) and the Madeiran archipelago plants produced exclusively short-dispersible diaspores. Genetically, the studied populations fell into four main island groups: Lanzarote–Fuerteventura, Gran Canaria, Tenerife–El Hierro and La Gomera–Madeira archipelago.
- **Conclusions** A Moroccan origin of *R. bucephalophorus* subsp. *canariensis* is hypothesized with a colonization route from the eastern to the western islands. In addition, at least one gene flow event from La Gomera to the Madeiran archipelago has taken place. During the colonization process the type of dispersible diaspore changed so that dispersability decreased in populations of the westernmost islands.

Key words: *Rumex bucephalophorus* subsp. *canariensis*, Polygonaceae, Macaronesia, Canary archipelago, Madeiran archipelago, AFLP, heterocarpy, colonization events, diaspore polymorphism.

INTRODUCTION

In recent decades, oceanic archipelagos have been identified as ‘living laboratories’ that are optimal for the study of the evolutionary processes and patterns of diversification of lineages of plants (Crawford *et al.*, 1987; Andersen, 1995; Weller *et al.*, 1996; Crawford and Stuessy, 1997; Kim *et al.*, 2008) and animals (Desalle and Templeton, 1988; Emerson *et al.*, 1999; Juan *et al.*, 2000). A general pattern on oceanic islands is a high frequency of endemism in comparison with large continental areas (Sanmartín *et al.*, 2008). Isolation and marked habitat diversity give rise to adaptive radiation than in turn leads to high levels of endemism (Crawford *et al.*, 1987; Kim *et al.*, 1996; Baldwin *et al.*, 1998; Barber *et al.*, 2002; Fuertes-Aguilar *et al.*, 2002; Allan *et al.*, 2004; Trusty *et al.*, 2005; Goodson *et al.*, 2006; Garnatje *et al.*, 2007; Sanmartín *et al.*, 2008; see review in Caujapé-Castells, 2011).

The Canary Islands exhibit one of the highest percentages of endemic taxa (Francisco-Ortega *et al.*, 2000; Juan *et al.*, 2000; but see Keeley and Funk, 2011) and, although some authors have considered some of these to be relicts of Tertiary origin, recent molecular data reveal a common pattern of dispersion from the mainland followed by speciation (Böhle

et al., 1996; Kim *et al.*, 1996; Francisco-Ortega *et al.*, 1995a, b, 1996a, b, 1997). Given the prevailing winds and sea currents, two plausible sources of colonizers are neighbouring North Africa and the Iberian Peninsula (Juan *et al.*, 2000). The colonizers from Africa are more likely to arrive first on the easternmost islands, but the likely paths for Iberian colonizers are less obvious. Molecular phylogenetic analyses of animals and plants from the Canary Islands have shown that the predominant pattern is a stepwise colonization from the older islands in the east to the younger islands in the west of the archipelago (Juan *et al.*, 2000). During such colonization, a decrease in genetic diversity from older to more recently established populations would be expected (Hedrick, 1999; Maki, 2001; García-Verdugo *et al.*, 2009). However, the pattern of colonization may be complicated by different factors, such as back colonization, extinction or multiple colonization events (Juan *et al.*, 2000; Emerson, 2002).

Rumex bucephalophorus is a Mediterranean species that began to diversify around 4.2 Mya (Talavera *et al.*, 2011). According to Press (1988) it has four subspecies: *R. bucephalophorus* subsp. *bucephalophorus*, *R. bucephalophorus* subsp. *gallicus*, *R. bucephalophorus* subsp. *hispanicus* and *R. bucephalophorus* subsp. *canariensis*. Of these only *R. bucephalophorus* subsp.

canariensis is endemic to Madeira and the Canary archipelagos (Hansen and Sunding, 1979) and is the youngest subspecies (<1 Myr; Talavera et al., 2011). *Rumex bucephalophorus* subsp. *hispanicus* occurs mainly in the north-west Iberian Peninsula and south-west France (López González, 1990) and also in the Azores (Hansen and Sunding, 1979), *R. bucephalophorus* subsp. *gallicus* extends from the Mediterranean basin to the Atlantic coast of the Iberian Peninsula and North Africa and *R. bucephalophorus* subsp. *bucephalophorus* occurs only on the shores of the Mediterranean basin (Press, 1988).

Rumex bucephalophorus is a heterocarpic species that produces up to four types of diaspores differing in morphology, mass and, as recently established, also in dispersability (Talavera et al., 2012). Plants can produce buried non-dispersible diaspores (BD), aerial fixed non-dispersible diaspores (FD), aerial short-dispersible diaspores (SD) and aerial long-dispersible diaspores (LD) (Talavera et al., 2010, 2011). The two dispersible diaspores differ in mass, terminal velocity and dispersal distance, with LD achieving greater distances than SD (Talavera et al., 2012). Diaspore polymorphisms that influence dispersal rates can have important effects on the phylogeographical lineages found on islands, and it has been claimed that dispersal capacity is an important factor affecting the genetic diversity of lineages, with low dispersal ability enhancing the propensity for speciation (Papadopoulou et al., 2009).

In *R. bucephalophorus*, the presence of the different types of diaspores has been traditionally used to characterize the four subspecies (Press, 1988), with *R. bucephalophorus* subsp. *canariensis* and *R. bucephalophorus* subsp. *hispanicus* having only SD. However, an extensive survey of populations of *R. bucephalophorus* subsp. *canariensis* across the Canary and Madeiran archipelagos has shown the existence of the other diaspore types (BD, FD and LD) in this taxon (Talavera et al., 2012). These three diaspore types also occur in *R. bucephalophorus* subsp. *gallicus*.

Our primary interest was to clarify the origin, or origins, of *R. bucephalophorus* subsp. *canariensis* in Macaronesia and to infer the colonization sequence among the islands. The high diversity of diaspore types in the Macaronesian populations could indicate the existence of multiple colonization events. However, a single colonization event followed by a rapid diversification may also have occurred, possibly as a consequence of selective pressures suffered by the plants on these islands. Specifically, we address the following questions: (1) Can we determine the route of entry into Macaronesia? (2) Can we infer the route or routes of the subsequent colonization to the different islands? (3) Has this subspecies experienced changes in diaspore type during colonization? To attempt to answer these questions, we used amplified fragment length polymorphism (AFLP) markers to infer phylogeographical relationships among populations and we have recorded the types of diaspores in the studied populations.

MATERIALS AND METHODS

Study area

The Canary and Madeiran archipelagos are part of Macaronesia, a biogeographical region located in the eastern Atlantic Ocean

between 40° and 15°N (Fig. 1). These archipelagos consist of three (Madeira) and seven (Canaries) islands that originated from volcanic activity during the Tertiary (Carracedo, 1999; Geldmacher et al., 2000; Valadão et al., 2002). The Canary Islands are aligned from east to west, with the easternmost, i.e. those nearest to the African mainland, being the oldest, and the more distant western islands the youngest (Fig. 1; Coello et al., 1992). Islands of the Madeiran archipelago are situated about 400 km north from the Canary archipelago and again the easternmost island is the oldest.

Study taxon

Rumex bucephalophorus subsp. *canariensis* is an annual pioneer species that varies markedly in size, although plants of the highlands of Madeira are perennial and suffruticose (*R. bucephalophorus* subsp. *canariensis* var. *fruticescens*). In the eastern islands of the Canary archipelago (Lanzarote and Fuerteventura), populations have colonized the dry, volcanic substrates to form part of the sparse herbaceous community. On the other islands of the Canary and Madeiran archipelagos, populations live from near sea level to 1720 m as part of open scrub, grasslands or mountain habitats and even in wet laurel forests.

For molecular analyses we used 22 populations of *R. bucephalophorus* subsp. *canariensis*: four from the Madeiran archipelago (Fig. 1A) and 18 from the Canary archipelago (Fig. 1B). Four additional populations of *R. bucephalophorus* were used as outgroups: one of *R. bucephalophorus* subsp. *hispanicus* from north-west Spain (Lugo, 43°33'N, 7°11'W; outgroup 4 in Fig. 2A) and three of *R. bucephalophorus* subsp. *gallicus*, two from Morocco (Taradant, 30°51'N, 8°21'W and Larache, 35°08'N, 6°08'W; outgroups 1 and 2 in Fig. 2A, respectively) and one from southern Spain (Cadiz, 36°11'N, 5°57'W; outgroup 3 in Fig. 2A). Fresh leaves from 12 individuals were collected from each population, apart from one population from Madeira and one from Fuerteventura (11 and nine samples, respectively). In populations used as outgroups five plants were sampled. Leaves were dried with silica gel for further analysis in the laboratory. The total number of samples was 280 (260 ingroups and 20 outgroups).

DNA extraction and AFLP protocol

Total genomic DNA was extracted from the 280 individuals with a plant extraction kit (DNeasy Plant Mini Kit from Qiagen, Valencia, CA, USA) following the manufacturer's protocol. The amount of DNA isolated was checked on a 1% agarose gel (0.5× TBE), and the mean concentration of the DNA was estimated photometrically (Specgene; Techne, Stone, UK).

Approximately 200 ng of dried material per sample was used in the AFLP analysis, following the protocols established by Vos et al. (1995). Genomic DNA was digested with two restriction endonucleases (*EcoRI* and *MseI*), the fragments ligated to double stranded adaptors (*EcoRI* and *MseI*) at 37°C for 2 h, and then diluted 20-fold with TE_{0.1} buffer. Fragments with matching nucleotides were amplified (downstream of the restriction sites) using pre-selective primers based on *EcoRI* and *MseI* adaptors. Pre-selective and selective

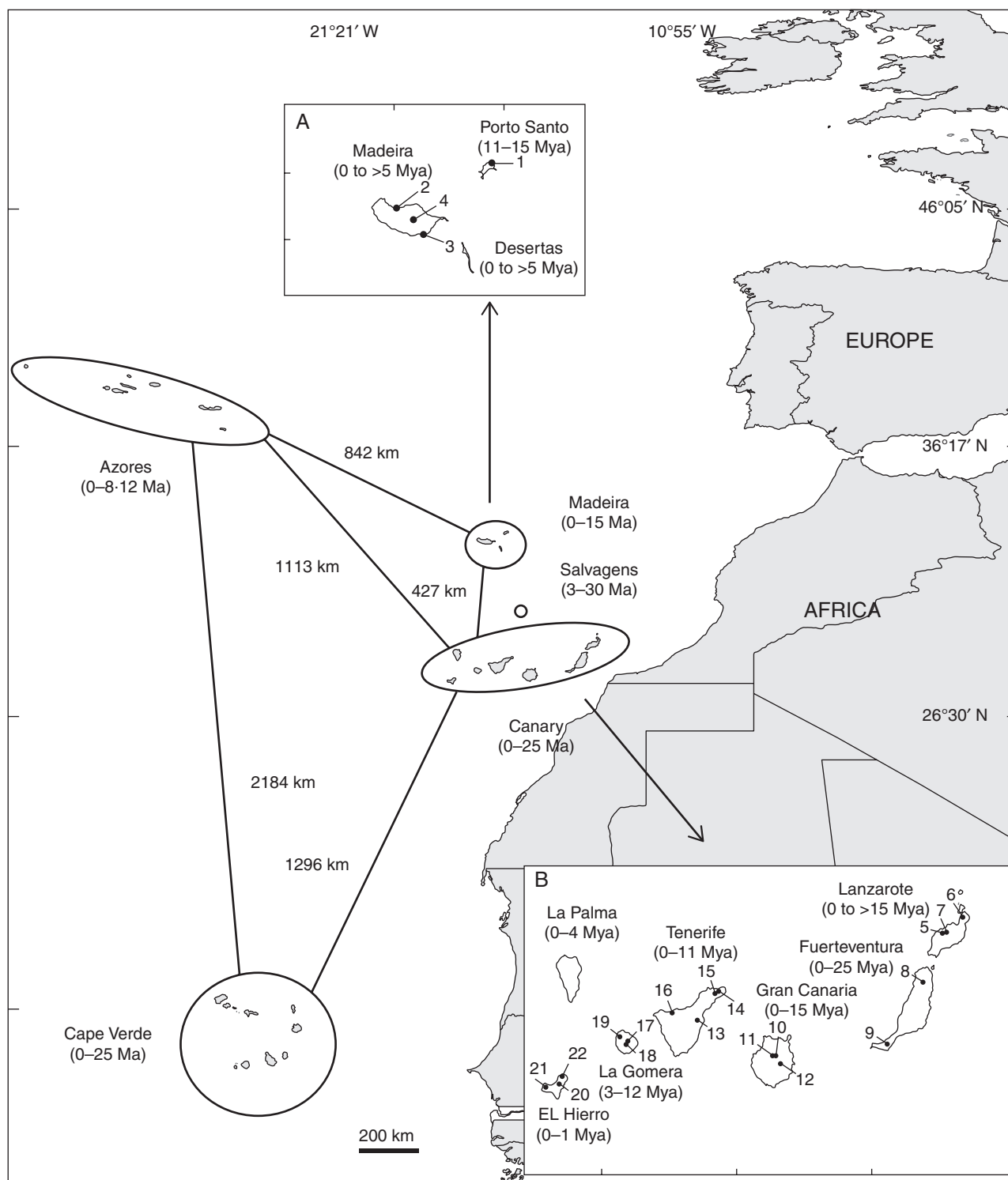


FIG 1. The Macaronesian region. For each archipelago, the period of formation is indicated in parentheses; zero indicates volcanic eruptions that have continued through historical times. The minimum distance separating the islands from each other is also indicated. (A) The locations of the four sampled populations of *Rumex bucephalophorus* subsp. *canariensis* in the Madeiran archipelago. (B) The locations of the sampled populations of *Rumex bucephalophorus* subsp. *canariensis* in the Canary archipelago.

amplifications were performed in a thermal cycler (Veriti 96 Well Thermal Cycler; Applied Biosystems, Foster City, CA, USA). To assess the reliability of the method, a random

fraction ($n = 22$, i.e. 8.3%) of the samples was replicated. It was found that duplicate analyses were largely indistinguishable, with a 98% repeatability of bands. Of the 36 primer

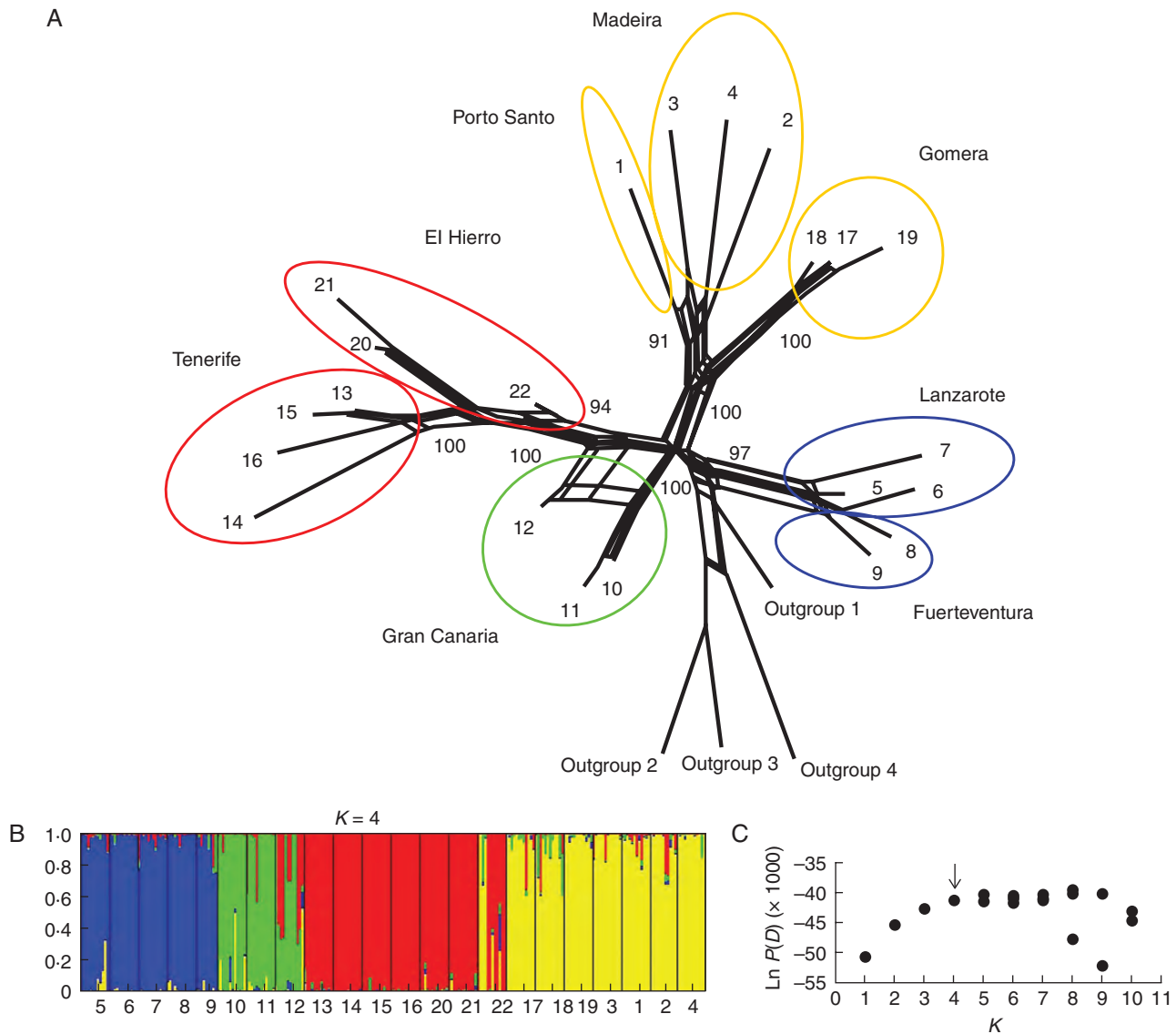


FIG. 2. (A) The tree generated by SplitsTree composition, based on the genetic distances of Nei and Li, for 22 populations of *Rumex bucephalophorus* subsp. *canariensis*, and four populations of *R. bucephalophorus* that are representatives of the two closest subspecies (*R. bucephalophorus* subsp. *hispanicus* and *R. bucephalophorus* subsp. *gallicus*). The population numbers correspond to those in Table 1. The branches indicate the value of the support (10 000 replicates) if $>50\%$. (B) The genetic structure of *Rumex bucephalophorus* subsp. *canariensis* inferred by Bayesian clustering of AFLP data, with assignment of 260 K into individual genetically distinguishable groups. Each individual is represented by a coloured vertical bar assigned according to the group(s). Populations are identified in Table 1. The band shows the most stable and likely assignment estimated by the structure (at $K = 4$) (see text). (C) Naperian logarithm of the posterior probability of the AFLP data, $\ln P(D)$, according to the different possible clusters (K) in *R. bucephalophorus* subsp. *canariensis*. The most probable K value is indicated by an arrow.

combinations tested in eight populations, three were selected because of their marked polymorphism: EcoRI-CAA/MseI-ACT (FAM), EcoRI-CAT/MseI-ACG (VIC) and EcoRI-CTG/MseI -AAC (NED). The fluorescently labelled products of the selective amplification phase were separated on a capillary automated sequencer (ABI 3730 DNA Analyzer; Applied Biosystems), with an internal size standard (GeneScan[®]-500 LIZ; Applied Biosystems) at the Genomics Unit of the Universidad Complutense of Madrid (Spain). The DNA fragments generated were aligned by molecular weight using the size standard and analysed with GeneMark 1.9 (SoftGenetics, LLC, State College, PA, USA). To avoid homoplasy, the

fragments of low molecular weight (<75 bp) were discarded, and the analysis was performed with well-defined fragments with lengths between 75 and 500 bp. The results were summarized in a presence/absence matrix.

Statistical analysis

As measures of diversity within populations, the following parameters were calculated: (1) the number of polymorphic fragments ($Frag_{poly}$) was calculated using the program FAMD 1.08 (Schlüter and Harris, 2006); (2) Nei's mean genetic diversity index (H_D) was obtained with the program

TABLE 1. Location, genetic diversity parameters and type of dispersible diaspore of the studied populations of *Rumex bucephalophorus subsp. canariensis*

Archipelago/Island/Population	Elevation (m)	Coordinates (N/W)	Collector	AFLP						Diaspores			
				<i>n</i>	Frag _{poly}	<i>H_D</i>	Frag _{priv}	DW	<i>F_{ST}</i>	SD (%)	LD (%)	<i>n</i>	
a. Archipelago of Madeira													
1. Porto Santo. Pico das Urzes	280	33°06'/16°19'	MS	12	158	0·07	8	25·78			100	0	12
2. Madeira I. Seixal	60	32°48'/17°04'	MS	11	140	0·06	4	19·25			100	0	27
3. Madeira II. Garajau	170	32°38'/16°51'	MS	12	136	0·06	4	19·61			100	0	12
4. Madeira III. Pico Arieiro*	1720	32°44'/16°56'	MS	12	162	0·07	4	25·33			100	0	50
Mean					146	0·06	4	21·40	0·22				
b. Archipelago of Canary													
5. Lanzarote I. Caldera Blanca	150	29°03'/13°43'	RB	12	241	0·10	11	45·46			0	100	47
6. Lanzarote II. Monte Corona	409	29°11'/13°29'	MT, RS	12	206	0·09	9	37·52			0	100	33
7. Lanzarote III. Montaña Tinache	346	29°03'/13°40'	MT, RS	12	198	0·08	5	30·18			0	100	20
Mean					215	0·09	8·33	37·72	0·08				
8. Fuerteventura I. La Oliva	272	28°38'/13°56'	MT, RS	12	188	0·08	1	28·05			0	100	21
9. Fuerteventura II. Bco de Esquinzo	256	28°06'/14°20'	MT, RS	9	255	0·11	10	42·32			0	100	9
Mean					221	0·10	5·50	35·19	0·11				
10. Gran Canaria I. Vega de San Mateo	1235	28°00'/15°34'	MT, PO, MA, AT	12	235	0·09	19	50·01			0	100	22
11. Gran Canaria II. Cruz de Tejeda	1488	28°00'/15°36'	MT, PO, MA, AT	12	247	0·10	9	45·80			0	100	19
12. Gran Canaria III. Bco de Guayadeque	907	27°56'/15°31'	MT, PO, MA, AT	12	217	0·09	11	36·27			0	100	14
Mean					233	0·09	13·00	44·03	0·09				
13. Tenerife I. B. de Badajoz	579	28°18'/16°26'	MT, PO, MA, AT	12	211	0·08	3	31·85			100	0	22
14. Tenerife II. Anaga	577	28°33'/16°12'	MT, PO, MA, AT	12	146	0·07	0	17·59			100	0	12
15. Tenerife III. Las Casas de la Cumbre	837	28°32'/16°14'	MT, PO, MA, AT	12	197	0·08	4	30·09			100	0	12
16. Tenerife IV. Icod de los Vinos	447	28°22'/16°43'	MT, PO, MA, AT	12	182	0·07	4	27·38			100	0	12
Mean					184	0·08	2·75	26·73	0·12				
17. La Gomera I. El Cedro	1096	28°07'/17°13'	MA, PO	12	195	0·08	0	24·54			100	0	22
18. La Gomera II. Garajonay	1300	28°06'/17°14'	MA, PO	12	207	0·09	2	28·75			89	11	28
19. La Gomera III. Chorros de Epina	830	28°10'/17°18'	MA, PO	12	175	0·08	0	22·86			100	0	18
Mean					192	0·08	0·67	25·38	0·05				
20. El Hierro I. Mirador de Jinama	1221	27°46'/17°59'	MT, AT	12	221	0·09	7	38·07			100	0	23
21. El Hierro II. Ermita V. de los Reyes	682	27°44'/18°07'	MT, AT	12	210	0·08	6	33·52			100	0	21
22. El Hierro III. Mocanal	543	27°49'/17°56'	MT, AT	12	272	0·11	10	42·79			100	0	20
Mean					234	0·09	7·77	38·13	0·06				

n, number plants studied; AFLP: Frag_{poly}, number of polymorphic fragments; *H_D*, index of Nei's genetic diversity; Frag_{priv}, number of private fragments; DW, index of rarity; *F_{ST}*, fixation index among the populations within each island. The arithmetic means of these parameters are also shown for each island. Diaspores: SD, short-dispersible diaspore; LD, long-dispersible diaspore. * Population of var. *fruticescens*. Collector: AT, Anass Terrat; MA, Montserrat Arista; MS, Miguel Sequeira; MT, María Talavera; PO, Pedro L. Ortiz; RB, Regina Berjano; RS, Ramón Casimiro-Soriguer.

Arlequin 3·1 (Excoffier *et al.*, 2005); (3) the number of private fragments, i.e. the fragments that are unique to each population (Frag_{priv}), was calculated using the program FAMD 1·08 (Schlüter and Harris, 2006); and (4) the rate of rare fragments (DW), as applied to AFLP data by Schönswetter and Tribsch (2005), which is equivalent to the 'weighted-down-range' of Crisp *et al.* (2001), was calculated with the program AFLPdat (Ehrlich, 2006).

For each island, the fixation index derived from AMOVA (*F_{ST}*) was calculated to estimate the genetic distances averaged over all populations using the program Arlequin 3·1 (Excoffier *et al.*, 2005). We also estimated the number of exclusive shared fragments (i.e. fragments exclusively shared by a pair of

populations or islands that are not present in any other population or island) and the genetic distances (*F_{ST}*) between pairs of populations and between pairs of islands, the latter also being calculated using Arlequin 3·1.

The levels of contemporary gene flow were evaluated by testing for dominant marker mapping by the maximum-likelihood method with the program AFLPOP 1·1 (Duchesne and Bernatchez, 2002). To determine whether the AFLP data matrix yielded a statistically acceptable level of allocation, we assigned, prior to the test, 500 simulated genotypes that were created using the allele frequencies observed in each of the populations. Subsequently, these simulated genotypes were assigned to each of the 22 sampled populations. To test

the assignment of real genotypes, we gave the program the following assumptions: (1) allele frequencies of value 0 were replaced by $1/(\text{sample size} + 1)$, and (2) the minimum maximum-likelihood difference (MLD) to assign an individual to a population was set to 0 or 1. With $MLD = 0$, individuals were assigned to the population with the highest probability value. With $MLD = 1$, individuals were only assigned to a population if their probability of belonging to that population was at least ten times higher than the probability of their belonging to another population. When the probability of assigning an individual to any candidate population was below a certain threshold (in our case $P < 0.001$), we concluded that the individual did not belong to any of those populations (Duchesne and Bernatchez, 2002).

The correlations between genetic distances (F_{ST}) and geographical distances between populations were performed using a Mantel test based on a Spearman correlation with one million permutations (package *ade4* in R Statistical Software; Dray and Dufour, 2007). Given that most populations were from the Canary archipelago, and the only four Madeiran populations were distant from the remainder, we performed a Mantel test only for the Canary archipelago. In this test, all the populations of each island were considered as one metapopulation, and the shortest geographical distance in a straight line between each pair of islands was used.

The genetic relationships between the populations of *R. bucephalophorus* subsp. *canariensis* were represented by a model that was based on a dendrogram representation using the F_{ST} genetic distances (Arlequin 3.1) between the populations by the nearest neighbour method (neighbour-joining), with the population as the basic unit. The bootstrap of each node was tested with 10 000 pseudoreplicates using the program PAUP * 4.0 beta 10 (Swofford, 2002). We used the program 4.11.3 SplitsTree (Huson and Bryant, 2006) to generate the tree, which also allowed the outgroups to be freely positioned on the tree.

The genetic relationships between the populations were also studied with the Bayesian mapping technique in the program STRUCTURE 2.3.3 (Pritchard et al., 2000). The analysis was conducted using the mixed genotypes model ('admixture ancestry model'), in which each individual has the fraction corresponding to the other genetic groups observed, and other allele frequencies are correlated. To ensure the convergence of the Markov chain, 500 000 Monte Carlo iterations were performed, and 10 % of the initial data generated ('burn-in') was discarded. Each of the groups (K) from $K = 1$ to $K = 10$ was replicated between four and 13 times. To determine the number of genetic groups that was most likely, we took into account the following considerations: (1) the *a posteriori* probability of the Naperian logarithm of the data, $\ln P(D)$, and (2) the stability of the patterns of allocation throughout the different replicates. The genetic groups created for each value of K were also tested by an analysis of molecular variance (AMOVA) from $K = 1$ to $K = 8$ (total number of islands analysed).

Diaspore production

Diaspore production was analysed in 9–50 plants from each population used for AFLP analysis. One hundred and one plants from the Madeiran archipelago and 375 plants from the Canary

Islands were studied. For each plant the type of diaspore produced (BD, FD, SD or LD) was recorded. All studied material was deposited in the herbarium of the University of Seville (SEV).

RESULTS

AFLP

The 260 individuals analysed from *R. bucephalophorus* subsp. *canariensis* generated 771 fragments, of which 91 % were polymorphic. The three primers used produced the following fragment length polymorphisms: EcoRI-CAA/MseI-ACT, 232; EcoRI-CAT/MseI-ACG, 252; and EcoRI-CTG/MseI-AAC, 204. The repeatability of 32 individuals (four from each island) was 98 %.

Genetic diversity

In the Canary archipelago, most populations on the eastern islands (Lanzarote, Fuerteventura and Gran Canaria) showed higher genetic diversity than those of the western islands for all parameters measured (Table 1), with the exception of the populations from El Hierro. Populations from the Madeiran archipelago showed lower diversity parameters than those from the Canary archipelago (Table 1).

Analysis of the allocation of the genotypes (AFLPOP) of the 22 populations of *R. bucephalophorus* subsp. *canariensis* correctly assigned all of the individuals from the populations on the islands of Madeira to their own populations (Table 2). In contrast, for each population of the Canary archipelago, at least one individual was genetically more likely to belong to another population on the same island than to its own population (Table 2). The only exception was one population from Tenerife (pop. 16) where all individuals were assigned to their own population. The analysis also showed that on most of the Canary Islands there were individuals that were genetically matched to populations from other islands of the same archipelago. Only one individual, from a population on La Gomera (pop. 18) was matched to a population on the Madeiran island of Porto Santo (pop. 1; Table 2).

Genetic distances and exclusive shared fragments between populations and islands

In general, within each island, the genetic distance between populations was low ($F_{ST} \leq 0.2$) or very low ($F_{ST} < 0.05$), except among the populations on the island of Madeira, for which F_{ST} varied between 0.21 and 0.23 (Table 3). In contrast, between islands, the genetic distance between populations was high in most cases ($F_{ST} > 0.2$; Table 3).

In general, most populations did not share exclusive fragments or only shared one to three exclusive fragments (Table 3). Only two population pairs shared five exclusive fragments: Gran Canaria I–Gran Canaria II and Fuerteventura I–Lanzarote II.

Taking into account all populations of each island, the smallest genetic distances were between Lanzarote and Fuerteventura ($F_{ST} = 0.05$, Table 4) and between Tenerife and El Hierro ($F_{ST} = 0.12$). These pairs of islands also had the highest numbers of exclusive shared fragments: Lanzarote and Fuerteventura

TABLE 2. Results of the assignment of the genotypes (AFLPOP) of individuals to the populations of *Rumex bucephalophorus* subsp. canariensis

Donor	P.S.	Recipient																					
		Madeira				Lanzarote			Fuert.		G. Canar.			Tenerife				La Gomera			El Hierro		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
P.S.	1	11	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Madeira	2	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	1	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lanzarote	5	0	0	0	0	5	3	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	5	7	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0
	7	0	0	0	0	1	0	10	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Fuert.	8	0	0	0	0	0	2	1	10	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	9	0	0	0	0	1	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0
G. Canar.	10	0	0	0	0	0	0	0	0	0	7	4	0	0	0	0	0	0	0	0	0	0	0
	11	0	0	0	0	0	0	0	0	0	5	6	3	0	0	0	0	0	0	0	0	0	0
	12	0	0	0	0	0	0	0	0	0	0	2	8	0	0	0	0	0	0	0	0	0	1
Tenerife	13	0	0	0	0	0	0	0	0	0	0	0	0	7	0	6	0	0	0	0	2	0	0
	14	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0
	15	0	0	0	0	0	0	0	0	0	0	0	0	5	2	6	0	0	0	0	0	0	0
	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0	0
La Gomera	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	3	4	0	0	2
	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	6	0	0	0	0
	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	2	8	0	0	1
El Hierro	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	1	1
	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	8	6
	22	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	3	1

All of the populations were marked for each island (diagonally), and the individuals of a population that generally have a higher probability of being part of a population from a different island are marked in bold. P.S., Porto Santo; Fuert., Fuerteventura; G. Canar., Gran Canaria.

shared 30 of these fragments, and Tenerife and El Hierro shared 26 (Table 4). The largest genetic distance was found between Tenerife and La Gomera ($F_{ST} = 0.36$). In general, the genetic distances between Gran Canaria and all other islands were the smallest, whereas the highest were found between Tenerife and all other islands, except for El Hierro (Table 4).

The Mantel test showed that isolation of populations was correlated with geographical distance ($r = 0.3$, $P = 0.004$, $n = 18$). The same test at the island level showed no significant correlation between genetic distance and geographical distance in the Canary archipelago ($r = 0.19$, $P = 0.45$, $n = 6$).

Population groupings

Four strongly supported principal clades were distinguished with the SplitsTree composition (Fig. 2A). The first clade is formed by the populations from Lanzarote and Fuerteventura [with 97 % bootstrap support (BS)]; a second clade comprises the populations from Gran Canaria; a third clade is formed by the populations of the Madeira archipelago and also La Gomera (100 % BS); and the fourth clade includes the populations from El Hierro and Tenerife (100 % BS). In addition, in the SplitsTree dendrogram structure, the outgroup populations are located near the base of the clade formed by the populations of Fuerteventura and Lanzarote (Fig. 2A).

A Bayesian analysis performed with STRUCTURE shows that with $K = 4$, the populations were grouped as in the SplitsTree composition (Fig. 2B). With values of $K > 4$, the replicates became unstable, and most were in the same four

groups observed with $K = 4$. The figure generated with the Naperian logarithm of the likelihood of the data, $\ln P(D)$, showed that the most likely number of clusters was four; with higher K values the different replicates were unstable (Fig. 2C).

AMOVA showed that, in the overall analysis ($K = 1$), 71.26 % of the genetic variation among the plants occurred within the populations, with 28.74 % among the populations. The percentage of molecular variance, assuming the classification into phenetic groups defined by the other methods of management (SplitsTree composition, STRUCTURE), i.e. $K = 4$, was 20.89 % among groups, 11.40 % between populations within groups and 67.71 % among individuals within populations. Considering each island as a group ($K = 8$), 22.29 % of the molecular variation was among the islands, 7.97 % among populations within islands and 69.74 % among individuals within populations; these proportions were similar to those obtained with $K = 6$ and $K = 7$.

Diaspore production

Diaspore production was markedly variable among plants and ranged from 22 to 1764 (446.82 ± 37.8 ; mean \pm s.e.). All studied plants produced aerial fixed non-dispersible diaspores (FD) and a sole type of dispersible diaspore (SD or LD). Dispersible diaspores represented more than 90 % of the total production. Buried non-dispersible diaspores (BD) were very infrequent and they were found in only five plants. In the Canary archipelago, all populations from the

TABLE 3. Number of exclusive shared fragments, above the diagonal, and the genetic distance, below the diagonal, among populations of *Rumex bucephalophorus* subsp. *canariensis*

Porto	Madeira			Lanzarote			Fuertevent.		Gran Canaria			Tenerife				La Gomera			El Hierro			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	–	0	1	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0
2	0.21	–	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
3	0.18	<i>0.23</i>	–	1	0	0	0	0	2	0	0	0	0	1	0	0	0	1	0	0	0	0
4	0.23	<i>0.21</i>	<i>0.21</i>	–	0	0	0	0	1	2	1	0	0	1	0	1	0	0	0	0	0	1
5	0.27	0.31	0.28	0.31	–	0	1	2	1	0	1	0	1	0	0	0	2	1	1	2	2	0
6	0.31	0.34	0.31	0.33	<i>0.05</i>	–	1	5	0	1	0	0	0	0	1	0	1	0	0	0	1	2
7	0.34	0.34	0.35	0.37	<i>0.08</i>	<i>0.12</i>	–	0	2	0	0	0	0	0	0	0	0	0	0	1	0	0
8	0.31	0.35	0.33	0.36	0.10	0.07	0.14	–	2	0	1	0	1	0	0	0	0	0	0	0	0	0
9	0.28	0.31	0.30	0.32	0.07	0.07	0.10	<i>0.10</i>	–	0	2	0	0	1	1	1	0	0	0	0	0	0
10	0.23	0.27	0.28	0.29	0.19	0.21	0.23	0.25	0.19	–	5	2	1	0	0	0	0	1	1	0	1	0
11	0.23	0.28	0.27	0.30	0.23	0.24	0.26	0.27	0.20	<i>0.03</i>	–	3	1	1	0	0	0	0	1	0	0	0
12	0.26	0.29	0.30	0.32	0.22	0.26	0.24	0.28	0.22	<i>0.11</i>	<i>0.11</i>	–	1	0	0	0	0	1	0	0	0	0
13	0.35	0.38	0.39	0.42	0.30	0.34	0.33	0.35	0.29	0.27	0.27	0.20	–	0	1	0	0	0	0	0	0	0
14	0.42	0.44	0.46	0.47	0.38	0.42	0.41	0.43	0.37	0.35	0.36	0.27	<i>0.15</i>	–	0	0	0	1	0	1	0	0
15	0.37	0.40	0.42	0.44	0.34	0.37	0.37	0.38	0.32	0.31	0.30	0.23	<i>0.03</i>	<i>0.14</i>	–	1	0	0	0	1	1	0
16	0.40	0.43	0.44	0.45	0.36	0.41	0.38	0.41	0.35	0.34	0.34	0.23	<i>0.11</i>	<i>0.20</i>	<i>0.11</i>	–	0	0	0	0	1	1
17	0.23	0.25	0.24	0.20	0.26	0.29	0.32	0.32	0.28	0.20	0.22	0.27	0.36	0.42	0.39	0.41	–	0	2	0	0	0
18	0.23	0.24	0.23	0.21	0.24	0.28	0.31	0.30	0.26	0.18	0.21	0.25	0.34	0.42	0.38	0.40	<i>0.04</i>	–	1	0	0	0
19	0.26	0.29	0.27	0.25	0.26	0.30	0.33	0.33	0.28	0.24	0.26	0.30	0.40	0.45	0.43	0.44	<i>0.04</i>	<i>0.08</i>	–	0	0	0
20	0.36	0.37	0.39	0.41	0.31	0.35	0.32	0.37	0.30	0.29	0.29	0.22	0.13	0.21	0.15	0.18	0.37	0.37	0.41	–	1	0
21	0.39	0.40	0.42	0.43	0.34	0.38	0.37	0.41	0.34	0.32	0.33	0.26	0.18	0.27	0.19	0.20	0.39	0.39	0.43	<i>0.05</i>	–	1
22	0.20	0.23	0.24	0.25	0.18	0.23	0.21	0.25	0.19	0.16	0.18	0.13	0.11	0.20	0.14	0.15	0.19	0.20	0.23	<i>0.07</i>	<i>0.07</i>	–

The genetic distances between populations on the same island are indicated in italics, and F_{ST} distances <0.2 between populations of different islands are marked in bold. Porto, Porto Santo; Fuertevent., Fuerteventura.

TABLE 4. Number of exclusive shared fragments, above the diagonal, and the genetic distance, below the diagonal, between islands (considering all of the individuals of the island as a metapopulation) of *R. bucephalophorus* subsp. *canariensis*

	Porto Santo	Madeira	Lanzarote	Fuerteventura	Gran Canaria	Tenerife	Gomera	Hierro
Porto Santo	–	1	0	2	2	0	1	1
Madeira	0.13	–	0	3	3	5	4	1
Lanzarote	0.26	0.25	–	30	7	2	9	11
Fuerteventura	0.27	0.27	0.05	–	5	4	1	1
Gran Canaria	0.19	0.21	0.19	0.21	–	14	9	6
Tenerife	0.33	0.34	0.32	0.33	0.24	–	4	26
Gomera	0.21	0.16	0.26	0.28	0.20	0.36	–	7
Hierro	0.28	0.28	0.26	0.29	0.20	0.12	0.31	–

most eastern islands (Lanzarote, Fuerteventura and Gran Canaria) produced LD diaspores, while those from the remaining islands produced SD diaspores. The only exception was the Garajonay population from La Gomera where three plants produced LD diaspores and 25 plants produced SD diaspores. In the Madeira archipelago, all populations produced SD diaspores (Table 1).

DISCUSSION

Genetic diversity

Rumex bucephalophorus subsp. *canariensis* populations showed low or very low genetic diversity (H_D) compared with many of the mainland populations of this species (Talavera et al., 2011). These results are consistent with those found in most species endemic to these islands, in plants (Garnatje et al., 1998; Francisco-Ortega et al., 2000) and animals (Frankham, 1997, 1998; Brown and Pestano, 1998). The low genetic diversity exhibited by such island populations compared with those from the mainland has been attributed to founder effects and to the restricted areas usually found on islands (Frankham, 1997, 1998; Maki, 2001; Nielsen, 2004; García-Verdugo et al., 2009). Moreover, in *R. bucephalophorus* subsp. *canariensis*, we found a geographical pattern, with higher genetic diversity in the older, eastern islands than in the younger, western ones, and this could be a consequence of an east–west colonizing sequence of *R. bucephalophorus* subsp. *canariensis* in these islands, as has been reported for other taxa from the Canary archipelago, e.g. in *Olea europaea* subsp. *guanchica* (García-Verdugo et al., 2009).

According to the mapping analysis of individuals of *R. bucephalophorus* subsp. *canariensis* (AFLPOP), a common feature in most populations was the presence of immigrants from other populations (Table 2), except the Madeiran populations. Generally, gene flow was restricted to populations within the same island but with three main exceptions: Lanzarote, Fuerteventura and El Hierro. Populations from Lanzarote and Fuerteventura showed high gene flow between them. It was especially high in Lanzarote I and Fuerteventura II, two populations that also showed high genetic diversity. Gene flow between Lanzarote and Fuerteventura is likely to occur because they are separated by only 10.6 km and populations in these islands produce LD diaspores that are dispersed over long distances by wind. These two islands are dominated by sands and rocky plains with sclerophyll and thorny vegetation in which anemochorous dispersal may be favoured and trigger

gene flow. Moreover, these two islands were united until around 10 000 years ago (Machado, 1979), further enhancing possible gene flow. The other exception is El Hierro, more specifically the population El Hierro III, which comprised a mixture of genetically very distinct individuals that were seemingly derived from populations on other islands and from other populations on El Hierro (Table 2). Natural gene flow is a possible explanation, but we cannot exclude anthropogenically mediated migration because El Hierro III is located on the edge of the main road on the island.

Gene flow between populations on the island of Madeira was absent and consequently the genetic distance between them was relatively high ($F_{ST} > 0.2$). The terrain on Madeira is steep, and the populations studied are separated by the mountain range that crosses the island. Therefore, despite their proximity, the populations appear to be reproductively isolated. The Madeira III population consists of suffruticose plants (var. *fruticescens*). The fact that the montane population Madeira III has established, presumably *in situ*, a suffruticose habit, and appears to have been isolated for the last 0.3 Myr (Talavera et al., 2011), strongly supports the reproductive isolation of these island populations. However, Madeira II had two individuals that were genetically more similar to those of the population on Porto Santo, which is separated from Madeira by 40 km.

Phylogeography

All analyses performed on *R. bucephalophorus* subsp. *canariensis* have shown that the 22 populations could be arranged into four main groups: Lanzarote–Fuerteventura, Gran Canaria, Tenerife–El Hierro and La Gomera–Madeira archipelago (Fig. 2).

The tree generated by SplitsTree composition shows that the arrangement of the four phenetic groups is radial, a configuration characteristic of trees of young taxa (Balao et al., 2010). In fact, *R. bucephalophorus* subsp. *canariensis* is a young taxon with an estimated age of <1 Myr (Talavera et al., 2011). Additionally, the same tree shows that *R. bucephalophorus* subsp. *gallicus* and *R. bucephalophorus* subsp. *hispanicus*, which are genetically close to *R. bucephalophorus* subsp. *canariensis* (Talavera et al., 2011), are connected to a branch of the Fuerteventura–Lanzarote group. This close relationship and the high genetic diversity found in Fuerteventura–Lanzarote indicate that *R. bucephalophorus* probably entered Macaronesia via these

two islands. This finding is unsurprising as during the Quaternary glacial periods, the distance of these islands from the African continent was <60 km (Carracedo *et al.*, 1998; García-Talavera, 1999), and according to Caujapé-Castells (2011), around 25% of the plants that colonized Macaronesia did so via this route.

However, the group comprising all populations from Gran Canaria were closer to the centre of the tree than the other two groups (Tenerife–El Hierro and La Gomera–Madeira archipelago). Among these three groups, Gran Canaria shows the highest genetic diversity and its genetic distance to either of the other two groups is lower than the distance between these two groups. These results indicate that, from Fuerteventura and Lanzarote, Gran Canaria, the nearest island to the west, was the next step in the colonization of the archipelago by *R. bucephalophorus*. Based on the topology of the tree generated by SplitsTree composition, from Gran Canaria we can infer that *R. bucephalophorus* subsp. *canariensis* passed to the neighbouring islands of Tenerife and La Gomera independently.

Although the nearest island to El Hierro is La Gomera, the average genetic distance between their populations was high ($F_{ST} = 3.1$), and Tenerife and El Hierro formed a single group. These results suggest that *R. bucephalophorus* subsp. *canariensis* reached El Hierro directly from Tenerife, avoiding an intermediate step on La Gomera. Surprisingly, the populations of La Gomera were closely related to populations of the Madeiran archipelago despite the great geographical distance between them. Indeed, all of the genetic criteria clearly showed that populations of *R. bucephalophorus* subsp. *canariensis* from Madeira and those from La Gomera form a phenetic group, indicating the existence of gene flow between them. In this group, populations from La Gomera showed higher genetic diversity than populations from Madeira (based on most parameters used), supporting the idea that migration occurred from the Canary islands towards the Madeiran archipelago. A close relationship between populations on the Canary islands and Madeira has been found in several phylogenetically distinct taxa [*Aeonium* alliance (Jorgensen and Olesen, 2001; Mort *et al.*, 2002; Fairfield *et al.*, 2004; Kim *et al.*, 2008), *Bystropogon* (Trusty *et al.*, 2005), *Convolvulus* (Carine *et al.*, 2004), *Crambe* (Francisco-Ortega *et al.*, 2002; Kim *et al.*, 2008), *Echium* (Kim *et al.*, 2008), *Pericallis* (Panero *et al.*, 1999), *Sideritis* (Kim *et al.*, 2008) and the woody *Sonchus* alliance (Kim *et al.*, 1996, 2008)], indicating that this pattern is not uncommon in central Macaronesia, and most of these studies revealed that populations from Madeira were derived from Canary populations.

Diaspore production

We found a clear geographical pattern of distribution of dispersible diaspore types. In the eastern Canary archipelago (Lanzarote, Fuerteventura and Gran Canaria), plants produce exclusively LD diaspores, whereas in the western Canary archipelago and Madeiran archipelago, plants produce exclusively SD diaspores. The only exception to this pattern was found in a population from La Gomera where plants with LD diaspores and plants with SD diaspores co-occur. The presence of a particular type of dispersible diaspore is a genetically

determined trait in *R. bucephalophorus* (Talavera *et al.*, 2012). Plants from the eastern Canaries have the same type of dispersible diaspore as plants of *R. bucephalophorus* subsp. *gallicus*, and the plants from the eastern islands are morphologically more similar to the Moroccan *R. bucephalophorus* subsp. *gallicus* plants than to those from the western islands. This fact reinforces the argument that entry of *R. bucephalophorus* subsp. *canariensis* took place via Fuerteventura–Lanzarote from Moroccan continental populations. Later, as colonization advanced to the western islands, the shift from producing LD diaspores to SD occurred at least twice (on Tenerife and La Gomera). However, because long-distance dispersal between islands is more likely to occur through LD diaspores, it is likely that the advance from Tenerife to El Hierro took place before the shift to SD occurred on the former island. The fact that LD diaspores are practically absent from western islands strongly suggests that long-distance dispersal has been negatively selected for in the more mesic environments of these islands. It has been proposed that on oceanic islands the cost of dispersal is high enough for selection to favour the reduction of dispersability (Carlquist, 1965; Cody and Overton, 1996; Fresnillo and Ehlers, 2008). As noted above, we found a lower genetic diversity in populations of westernmost islands, and this pattern is probably a consequence of the colonizing sequence. In *R. bucephalophorus* subsp. *canariensis*, the lower dispersability of populations on the western islands would constrain gene flow between them and consequently their genetic diversity. In conclusion, we have found an east-to-west colonization route in *R. bucephalophorus* in the Canary archipelago and the existence of gene flow between La Gomera and Madeira. As expected, we found a reduction of genetic diversity from the first colonized eastern islands to the later colonized western islands. Moreover, the type of dispersible diaspore changed during the colonization process so that dispersability decreased in populations of the westernmost islands.

ACKNOWLEDGEMENTS

This work was funded by FEDER funds allocated to projects from the Spanish Ministry of Science and Technology to P.L.O. (CGL2005-03 731, 02 533-E-CGL2008), to M.A. (CGL2008-02531-E; CGL2009-08257) and to S. Talavera (CGL2006-00817) and a PhD scholarship from the Spanish Ministry of Education and Science to M.T. We thank M. Sequeira, University of Madeira, O. Rodriguez, University of La Laguna (Tenerife), and R. Berjano, R. Casimiro-Soriguer and A. Terrab, all from the University of Seville, for their help in collecting materials. We also thank Dr P. E. Gibbs for revision of the English text and comments on the manuscript and the General Services of Herbarium and Biology (CITIUS, University of Seville) for allowing the use of their facilities.

LITERATURE CITED

- Allan GJ, Francisco-Ortega J, Santos-Guerra A, Boerner E, Zimmer EA. 2004. Molecular phylogenetic evidence for the geographic origin and classification of Canary Island *Lotus* (Fabaceae: Loteae). *Molecular Phylogenetics and Evolution* 32: 123–138.

- Andersen H. 1995. Research on islands: classic, recent and prospective approaches. In: Vitousek PM, Loope LL, Adersen H. eds. *Islands: biological diversity and ecosystem function*. Berlin: Springer, 7–21.
- Balao F, Valente LM, Vargas P, Herrera J, Talavera S. 2010. Radiative evolution of polyploid races of the Iberian carnation *Dianthus broteri* (Caryophyllaceae). *New Phytologist* **187**: 542–551.
- Baldwin BG, Crawford DJ, Francisco-Ortega J, Kim S-C, Sang T, Stuessy TF. 1998. Molecular phylogenetics insights on the origin and evolution of oceanic island plants. In: Soltis DE, Soltis PS, Doyle JJ. eds. *Molecular systematics of plants, II: ADN sequencing*. New York: Kluwer, 410–441.
- Barber JC, Francisco-Ortega J, Santos-Guerra A, Turner KG, Jansen RK. 2002. Origin of Macaronesian *Sideritis* L. (Lamiaceae: Lamiaceae) inferred from nuclear and chloroplast sequence datasets. *Molecular Phylogenetics and Evolution* **23**: 293–306.
- Böhle UR, Hilger HH, Martin WF. 1996. Island colonization and evolution of the insular woody habit in *Echium* L. (Boraginaceae). *Proceedings of the National Academy of Sciences of the USA* **93**: 11740–11745.
- Brown R, Pestano J. 1998. Phylogeography of skinks (Chalcididae) in the Canary Islands inferred from mitochondrial DNA sequences. *Molecular Ecology* **7**: 1183–1191.
- Carlquist SC. 1965. *Island life: a natural history of the islands of world*. Garden City, NY: Natural History Press.
- Carine MA, Russell SJ, Santos-Guerra A, Francisco-Ortega J. 2004. Relationships of the Macaronesian and Mediterranean floras: molecular evidence for multiple colonizations into Macaronesia and back-colonization of the continent in *Convolvulus* L. (Convolvulaceae). *American Journal of Botany* **91**: 1070–1085.
- Carracedo J. 1999. Growth, structure, instability and collapse of Canarian volcanoes and comparisons with Hawaiian volcanoes. *Journal of Volcanology and Geothermal Research* **94**: 1–19.
- Carracedo J, Day S, Guillou H, Rodríguez Badiola E, Canas J, Pérez Torrado F. 1998. Hotspot volcanism close to a passive continental margin: the Canary Islands. *Geological Magazine* **135**: 591–604.
- Caujapé-Castells J. 2011. Jesters, red queens, boomerangs and surfers: a molecular outlook on the diversity of the Canarian endemic flora. In: Bramwell D, Caujapé-Castells J. eds. *The biology of island floras*. Cambridge: Cambridge University Press, 284–324.
- Cody ML, McC Overton J. 1996. Short-term evolution of reduced dispersal in island plant populations. *Journal of Ecology* **84**: 53–61.
- Coello J, Cantagrel JM, Hernán F, et al. 1992. Evolution of the eastern volcanic ridge of the Canary Islands based on new K-Ar data. *Journal of Volcanology and Geothermal Research* **53**: 251–274.
- Crawford DJ, Stuessy TF. 1997. Plant speciation on oceanic islands. In: Iwatsuki K, Raven PH. eds. *Evolution and diversification in land plants*. Tokyo: Springer, 249–267.
- Crawford DJ, Whitkus R, Stuessy TF. 1987. Plant evolution and speciation on oceanic islands. In: Urbanska KM. ed. *Differentiation patterns in higher plants*. London: Academic Press, 183–199.
- Crisp M, Laffan S, Linder H, Monro A. 2001. Endemism in the Australian flora. *Journal of Biogeography* **28**: 183–198.
- Desalle R, Templeton AR. 1988. Founder effects and the rate of mitochondrial DNA evolution in Hawaiian *Drosophila*. *Evolution* **42**: 1076–1084.
- Dray S, Dufour A. 2007. The ade4 package: Implementing the duality diagram for ecologists. *Journal of Statistical Software* **22**: 1–20.
- Duchesne P, Bernatchez L. 2002. AFLPOP: a computer program for simulated and real population allocation, based on AFLP data. *Molecular Ecology Notes* **2**: 380–383.
- Ehrich D. 2006. AFLPdat: a collection of R functions for convenient handling of AFLP data. *Molecular Ecology Notes* **6**: 603–604.
- Emerson BC. 2002. Evolution on oceanic islands: molecular phylogenetic approaches to understanding pattern and process. *Molecular Ecology* **11**: 951–966.
- Emerson BC, Oromí P, Hewitt GM. 1999. MtDNA phylogeography and recent intra-island diversification among Canary Island Calathus beetles. *Molecular Phylogenetics and Evolution* **13**: 149–158.
- Excoffier L, Laval G, Schneider S. 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* **1**: 47–50.
- Fairfield KN, Mort ME, Santos-Guerra A. 2004. Phylogenetics and evolution of the Macaronesian members of the genus *Aichryson* (Crassulaceae) inferred from nuclear and chloroplast sequence data. *Plant Systematics and Evolution* **248**: 71–83.
- Francisco-Ortega J, Crawford DJ, Santos-Guerra A, Sa-Fontinha S. 1995a. Genetic divergence among Mediterranean and Macaronesian genera of the subtribe Crysanthemidae (Asteraceae). *American Journal of Botany* **82**: 1321–1328.
- Francisco-Ortega J, Jansen RK, Crawford DJ, Santos-Guerra A. 1995b. Chloroplast DNA evidence for intergeneric relationships of the Macaronesian endemic genus *Argyranthemum* (Asteraceae). *Systematic Botany* **20**: 413–422.
- Francisco-Ortega J, Crawford DJ, Santos-Guerra A, Carvalho JA. 1996a. Isozyme differentiation in the endemic genus *Argyranthemum* (Asteraceae: Anthemideae) in the Macaronesian Islands. *Plant Systematics and Evolution* **202**: 137–152.
- Francisco-Ortega J, Jansen RK, Santos-Guerra A. 1996b. Chloroplast DNA evidence of colonization, adaptive radiation, and hybridization in the evolution of the Macaronesian flora. *Proceedings of the National Academy of Sciences of the USA* **93**: 4085–4090.
- Francisco-Ortega J, Santos-Guerra A, Hines A, Jansen RK. 1997. Molecular evidence for a Mediterranean origin of the Macaronesian endemic genus *Argyranthemum* (Asteraceae). *American Journal of Botany* **84**: 1595–1613.
- Francisco-Ortega J, Santos-Guerra A, Kim S, Crawford D. 2000. Plant genetic diversity in the Canary Islands: a conservation perspective. *American Journal of Botany* **87**: 909–919.
- Francisco-Ortega J, Fuertes-Aguilar J, Kim SC, Santos-Guerra A, Crawford DJ, Jansen RK. 2002. Phylogeny of the Macaronesian endemic *Crambe* section *Dendrocrambe* (Brassicaceae) based on internal transcribed spacer sequences of nuclear ribosomal DNA. *American Journal of Botany* **89**: 1984–1990.
- Frankham R. 1997. Do island populations have less genetic variation than mainland populations? *Heredity* **78**: 311–327.
- Frankham R. 1998. Inbreeding and extinction: island populations. *Conservation Biology* **12**: 665–675.
- Fresnillo B, Ehlers BK. 2008. Variation in dispersability among mainland and island populations of three wind dispersed plant species. *Plant Systematics and Evolution* **270**: 243–255.
- Fuertes-Aguilar J, Ray M, Francisco-Ortega J, Santos-Guerra A, Jansen R. 2002. Molecular evidence from chloroplast and nuclear markers for multiple colonizations of *Lavatera* (Malvaceae) in the Canary Islands. *Systematic Botany* **27**: 74–83.
- García-Talavera F. 1999. La Macaronesia. Consideraciones geológicas, biogeográficas y paleoecológicas. In: Fernández-Palacios JM, Bacallado JJ, Belmonte JA. eds. *Ecología y cultura en Canarias*. Santa Cruz de Tenerife: Museo de la Ciencia y el Cosmos, Cabildo Insular de Tenerife, 39–63.
- García-Verdugo C, Fay MF, Granado-Yela C, et al. 2009. Genetic diversity and differentiation processes in the ploidy series of *Olea europaea* L.: a multiscale approach from subspecies to insular populations. *Molecular Ecology* **18**: 454–467.
- Garnatje T, Susanna A, Messeguer R. 1998. Isozyme studies in the genus *Cheirolophus* (Asteraceae: Cardueae-Centaureinae) in the Iberian Peninsula, North Africa and the Canary Islands. *Plant Systematics and Evolution* **213**: 57–70.
- Garnatje T, García S, Canela M. 2007. Genome size variation from a phylogenetic perspective in the genus *Cheirolophus* Cass. (Asteraceae): biogeographic implications. *Plant Systematics and Evolution* **264**: 117–134.
- Geldmacher J, van den Bogaard P, Hoernle K, Schmincke H. 2000. The ⁴⁰Ar/³⁹Ar age dating of the Madeira Archipelago and hotspot track (eastern North Atlantic). *Geochemistry Geophysics Geosystems* **1**: 1–26.
- Goodson B, Santos-Guerra A, Jansen R. 2006. Molecular systematics of *Descurainia* (Brassicaceae) in the Canary Islands: biogeographic and taxonomic implications. *Taxon* **55**: 671–682.
- Hansen A, Sunding P. 1979. *Flora of Macaronesia. Checklist of vascular plants*. 2nd edn. Oslo: Botanical Garden and Museum, University of Oslo.
- Hedrick PW. 1999. Perspective: highly variable loci and their interpretation in evolution and conservation. *Evolution* **53**: 313–318.
- Huson D, Bryant D. 2006. Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution* **23**: 254–267.
- Jorgensen TH, Olesen JM. 2001. Adaptive radiation of island plants: evidence from *Aeonium* (Crassulaceae) of the Canary Islands. *Perspectives in Plant Ecology, Evolution and Systematics* **4**: 29–42.
- Juan C, Emerson B, Oromí P, Hewitt G. 2000. Colonization and diversification: towards a phylogeographic synthesis for the Canary Islands. *Trends in Ecology & Evolution* **15**: 104–109.

- Keeley SC, Funk VA. 2011.** Origin and evolution of Hawaiian endemics: new patterns revealed by molecular phylogenetic studies. In: Brawell D, Caujapé-Castells J. eds. *The biology of island floras*. Cambridge: Cambridge University Press, 57–88.
- Kim S, Crawford D, Francisco-Ortega J, Santos-Guerra A. 1996.** A common origin for woody *Sonchus* and five related genera in the Macaronesian islands: molecular evidence for extensive radiation. *Proceedings of the National Academy of Sciences* **93**: 7743–7748.
- Kim S, McGowen M, Lubinsky P, Barber J, Mort M, Santos-Guerra A. 2008.** Timing and tempo of early and successive adaptive radiations in Macaronesia. *PLoS One* **3**: e2139. <http://dx.doi.org/10.1371/journal.pone.0002139>.
- López González G. 1990.** Género *Rumex* L. In: Castroviejo S, Laínz M, López González G, et al eds. *Flora iberica*. Madrid: Real Jardín Botánico, C.S.I.C., 595–634.
- Machado A. 1979.** Consideraciones sobre el género *Pimelia* (*Col. Tenebrionidae*) en las Islas Canarias y descripción de una nueva especie. *Boletín de la Asociación Española de Entomología* **3**: 119–127.
- Maki M. 2001.** Genetic differentiation within and among island populations of the endangered plant *Aster miyagii* (Asteraceae), an endemic to the Ryukyu Islands. *American Journal of Botany* **88**: 2189–2194.
- Mort ME, Soltis DE, Soltis PS, Francisco-Ortega J, Santos-Guerra A. 2002.** Phylogenies and evolution of the Macaronesian clade of Crassulaceae inferred from nuclear and chloroplast sequence data. *Systematic Botany* **27**: 271–288.
- Nielsen LR. 2004.** Molecular differentiation within and among island populations of the endemic plant *Scalesia affinis* (Asteraceae) from the Galápagos Islands. *Heredity* **93**: 434–442.
- Panero JL, Francisco-Ortega J, Jansen RK, Santos-Guerra A. 1999.** Molecular evidence for multiple origins of woodiness and a New World biogeographic connection of the Macaronesian Island endemic *Pericallis* (Asteraceae: Senecioneae). *Proceedings of the National Academy of Sciences of the USA* **96**: 13886–13891.
- Press JR. 1988.** Intraspecific variation in *Rumex bucephalophorus* L. *Botanical Journal of the Linnean Society* **97**: 344–355.
- Papadopoulou A, Anastasiou I, Keskin B, Vogler AP. 2009.** Comparative phylogeography of tenebrionid beetles in the Aegean archipelago: the effect of dispersal ability and habitat preference. *Molecular Ecology* **18**: 2503–2517.
- Pritchard JK, Stephens M, Donnelly P. 2000.** Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–959.
- Sanmartín I, van der Mark P, Ronquist F. 2008.** Inferring dispersal: a Bayesian approach to phylogeny-based island biogeography, with special reference to the Canary Islands. *Journal of Biogeography* **35**: 428–449.
- Schlüter PM, Harris SA. 2006.** Analysis of multilocus fingerprinting data sets containing missing data. *Molecular Ecology Notes* **6**: 569–572.
- Schönswetter P, Tribsch A. 2005.** Vicariance and dispersal in the alpine perennial *Bupleurum stellatum* L. (Apiaceae). *Taxon* **54**: 725–732.
- Swofford DL. 2002.** PAUP*: phylogenetic analysis using parsimony (*and other methods), Version 4. Sunderland, MA: Sinauer Associates.
- Talavera M, Ortiz PL, Arista M, Berjano R, Imbert E. 2010.** Disentangling sources of maternal effects in the heterocarpic species *Rumex bucephalophorus*. *Perspectives in Plant Ecology, Evolution and Systematics* **12**: 295–304.
- Talavera M, Balao F, Casimiro-Soriguer R, et al. 2011.** Molecular phylogeny and systematics of the highly polymorphic *Rumex bucephalophorus* complex (Polygonaceae). *Molecular Phylogenetics and Evolution* **61**: 659–670.
- Talavera M, Arista M, Ortiz PL. 2012.** Evolution of dispersal traits in a biogeographical context: a study using the heterocarpic *Rumex bucephalophorus* as a model. *Journal of Ecology* **100**: 1194–1203.
- Trusty J, Olmstead R, Santos-Guerra A, Sá-Fontinha S, Francisco-Ortega J. 2005.** Molecular phylogenetics of the Macaronesian endemic genus *Bystropogon* (Lamiaceae): palaeo-islands, ecological shifts and interisland colonizations. *Molecular Ecology* **14**: 1177–1189.
- Valadão P, Gaspar J, Queiroz G, Ferreira T. 2002.** Landslides density map of S. Miguel Island, Azores archipelago. *Natural Hazards and Earth System Sciences* **2**: 51–56.
- Vos P, Hogers R, Bleeker M, et al. 1995.** AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* **23**: 4407–4414.
- Weller SG, Sakai AK, Straub C. 1996.** Allozyme diversity and genetic identity in *Schiedea* and *Alsinidendron* (Caryophyllaceae: Alsinoideae) in the Hawaiian islands. *Evolution* **50**: 23–34.