Multiple Colonizations, In Situ Speciation, and Volcanism-Associated Stepping-Stone Dispersals Shaped the Phylogeography of the Macaronesian Red Fescues (*Festuca* L., Gramineae)

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Abstract.—Whereas examples of insular speciation within the endemic-rich Macaronesian hotspot flora have been documented, the phylogeography of recently evolved plants in the region has received little attention. The Macaronesian red fescues constitute a narrow and recent radiation of four closely related diploid species distributed in the Canary Islands (*F. agustinii*), Madeira (*F. jubata*), and the Azores (*F. francoi* and *F. petraea*), with a single extant relative distributed in mainland southwest Europe (*F. rivularis*). Bayesian structure and priority consensus tree approaches and population spatial correlations between genetic, geographical, and dispersal distances were used to elucidate the phylogeographical patterns of these grasses. Independent versus related origins and dispersal versus isolation by distance (IBD) hypotheses were tested to explain the genetic differentiation of species and populations, respectively. Genetic structure was found to be geographically distributed among the archipelagos and the islands endemics. The high number of shared AFLP fragments in all four species suggests a recent single origin from a continental Pliocene ancestor. However, the strong allelic structure detected among the Canarian, Madeiran, and Azorean endemics and the significant standardized residual values obtained from structured Bayesian analysis for pairwise related origin hypotheses strongly supported the existence of three independent continental-oceanic colonization events. The Canarian *F. agustinii*, the Madeiran *F. jubata*, and the two sister *F. francoi* and *F. petraea* Azorean species likely evolved from different continental founders in their respective archipelagos. Despite the short span of time elapsed since colonization, the two sympatric Azorean species probably diverged in situ, following ecological adaptation, from a common ancestor that arrived from the near mainland. Simple dispersal hypotheses explained most of the genetic variation at the species level better than IBD models. The optimal dispersal model for *F. agustinii* was a bidirectional centripetal stepping-stone colonization pattern, an eastern-to-western volcanism-associated dispersion was favored for *F. francoi*, whereas for the recently derived *F. petraea* a counterintuitive direction of colonization (west-to-east) was suggested. The population-based phylogeographical trends deduced from our study could be used as predictive models for other Macaronesian plant endemics with similar distribution areas and dispersal abilities. [Bayesian genetic analyses; colonization of oceanic islands; dispersal models; *Festuca* sect. *Aulaxyper*; Macaronesia; phylogeography.]

Oceanic islands have been considered natural laboratories for the study of colonization and evolutionary radiation processes (Darwin, 1859; Mayr, 1942; Carlquist, 1965) and for investigating biogeographical issues related to the origins and evolution of their respective biotas (MacArthur and Wilson, 1967; Emerson, 2002). Evolutionary theories about these islands that are often young in a geological context suggest that the potential for colonization is inversely related to isolation and that niche preemption often precludes multiple colonizations of congeneric taxa from the mainland (Whittaker, 1998; Silvertown, 2004; Carine et al., 2004). Modeling studies further suggest that species that locate few propagules in the dispersal medium with high survivorship for a given distance will ultimately reach the island(s) and undergo founder speciation (Paulay and Meyer, 2002).

The Macaronesian islands are characterized by a high level of plant endemism (Humphries, 1979; Bolos, 1996; Santos-Guerra, 1999) and the region represents one of the best-studied geographical settings of oceanic plant speciation (Francisco-Ortega et al., 1996; Moore et al., 2002; Carine et al., 2004). Most endemic Macaronesian lineages are derived clades and have sister clades that are distributed mainly in the western Mediterranean region (Emerson, 2002; Comes, 2004; Carine et al., 2004; Carine, 2005). The relatively short distance to the African and European continents would have allowed for the colonization from this area with the influence of

oceanic currents (North Atlantic and Canary streams) and northeasterly trade winds likely to have fostered the dissemination of plant germplasm from the continent. For this reason, it has been proposed that frequent colonizations from the continent have contributed to the high number of species endemic to the Canary Islands (Kim et al., 1999; Francisco-Ortega et al., 2000). For islands extremely isolated from continental sources, vacant ecological spaces are filled through adaptive radiation (Gillespie and Roderick, 2002). The origins of several Macaronesian plant groups have been interpreted as the results of single colonization events followed by in situ speciation (i.e., Böhle et al., 1996; Kim et al., 1996; Francisco-Ortega et al., 1997a, 1997b, 2002; Jorgensen and Frydenberg, 1999; Helfgott et al., 2000; Jorgensen and Olesen, 2001; Mort et al., 2002; reviewed in Silvertown, 2004, and Carine et al., 2004), though an increasing number of studies indicated the likely existence of multiple colonization events from the near continent (i.e., Francisco-Ortega et al., 1996; Panero et al., 1999; Vargas et al., 1999; Hess et al., 2000; Bohs and Olmstead, 2001; Percy and Cronk, 2002; Fuertes-Aguilar et al., 2002; Molero et al., 2002; reviewed in Silvertown, 2004, and Carine et al., 2004) or even from more distant regions (Carine, 2005). Genetic surveys that have explored the level and structure of genetic diversity of Macaronesian plant endemics have demonstrated greater levels of genetic variation than in Pacific oceanic endemic species
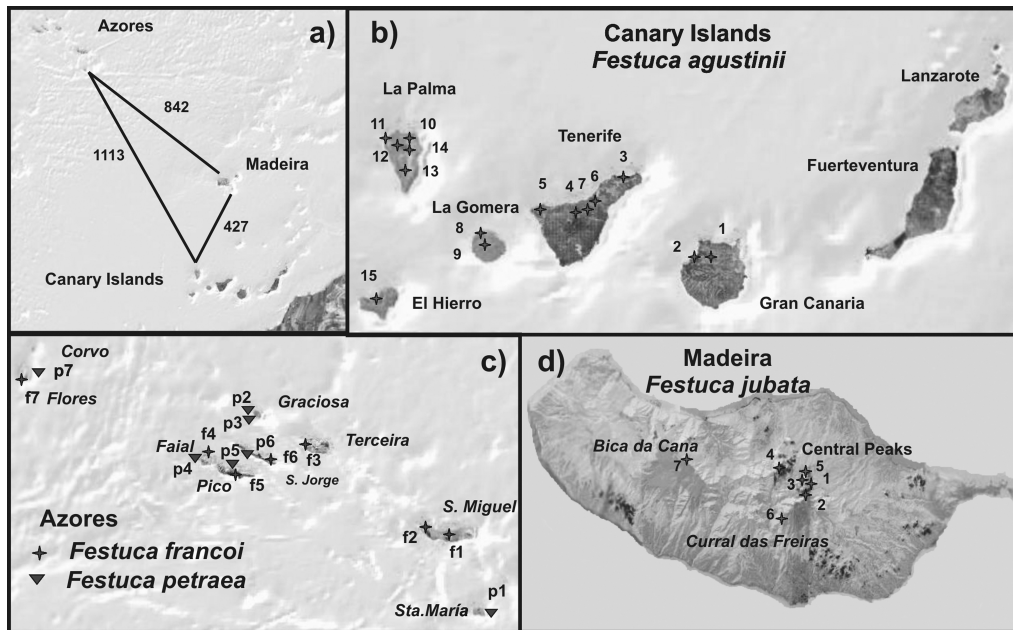
FIGURE 1. (a) Geographical distances (in km) among the three Macaronesian archipelagos containing the four native Macaronesian red fescues (*Festuca* sect. *Aulaxyper*) and (b–d) geographical location of their studied populations: (b) *F. agustinii* (western Canary isles, 15 populations); (c) *F. petraea* (Azores, 7 populations preceded by the letter p, triangles) and *F. francoi* (Azores, 7 populations preceded by the letter f, crosses); and (d) *F. jubata* (Madeira, 7 populations). Numbers are abbreviations from populations codes indicated in Table 1 (i.e., in the Canary Isles 1 identifies Fagus1).

(Frankham, 1997; Francisco-Ortega et al., 2000; Oliva et al., 2004), adding support to the evolutionary theory of higher feasibility of colonization from less distant main lands. However, recent molecular studies have found contrasting patterns of genetic diversity and genetic divergence among populations of different Macaronesian plant species (Francisco-Ortega et al., 2000; Batista and Sosa, 2002; Sánchez et al., 2004; Kim et al., 2005; Prohens et al., 2007).

Three successive stages of volcanic activity have been generally recognized in the geological evolution of Macaronesian islands: an initial shield-stage of high volcanism, followed by a quiescent stage, and ending with a stage of posterosional reduced volcanism (Carracedo, 1999; García-Talavera, 1999). This pattern is reproduced in the western Canary Isles where the oldest easternmost island of Gran Canaria (14.5 Ma) is presently in the final stage, the central Gomera (12 Ma) and Tenerife (7.5 Ma) islands are in the second, and the youngest westernmost islands of La Palma (2 Ma) and El Hierro (1.12 Ma) are in the first (Carracedo, 1999). The island of Madeira (5.3 Ma) is considered to be in a posterosional stage, which started approximately 0.7 Ma (Geldmacher et al., 2000). In contrast, most of the nine islands of the young archipelago of Azores (all except the oldest easternmost island of Santa Maria that is 8.12 Ma) show recent volcanic activity (Valadão et al., 2002). This occurs in all eastern (São Miguel, 4.01 Ma), central (Terceira, 3.52 Ma; Graciosa, 2.5 Ma; Faial, 0.73 Ma; São Jorge, 0.55 Ma; Pico, 0.23 Ma), and western (Flores, 2.16 Ma, Corvo, 0.71 Ma) subarchipelagos (Fig. 1). Explosive volcanic eruptions in

the Macaronesian islands have been linked with catastrophic demographic events that virtually eliminated all living organisms (Emerson, 2003). Massive landslides also affected the population genetic pools after important losses of islands volumes (Masson et al., 2002). Volcanic events have joined islands (e.g., Tenerife; São Miguel), favoring secondary contacts of previous allopatric populations, though more commonly they led to fragmentation of populations, resulting in new vicariant species (Gübbitz et al., 2005). All these phenomena, alone or in concert, have the potential to affect the phylogeography of the Macaronesian biotas.

The Macaronesian red fescues comprise four related species: *Festuca agustinii* Linding, *F. jubata* Lowe, *F. francoi* Fern. Prieto, C. Aguiar, E. Dias & M. I. Gut, and *F. petraea* Guthnick ex Seub. With the exception of *F. petraea*, which is found in coastal halophytic soils of the Azores, all species grow on medium- to high-altitude laurisilva cliffs in the central-western Canary Islands (Gomera, Gran Canaria, El Hierro, La Palma, Tenerife), Madeira, and Azores, respectively. Independent sources of evidence have suggested that these diploid species are of relative recent origin, as their lineages collapse in a basal polytomy, together with the only diploid continental species (*F. rivularis*) and an otherwise more recently evolved clade of highly polyploid cosmopolitan taxa, within the well supported *Festuca* sect. *Aulaxyper* (*F. rubra* group) clade (Catalán, 2006). A relaxed-clock analysis of nuclear ITS and plastid *trn*TF sequences of Loliinae suggest that the radiation occurred ca. 2.5 ± 0.9 Ma, assuming that the divergence between

Triticeae and Aveneae-Poeae ocurred ca. 21 Ma (Inda et al., 2008).

The simplest hypothesis for the colonization of Macaronesian archipelagos is that of a stepping-stone model of colonization (Emerson, 2002). A plausible scenario for the Canary Islands would involve the colonization of the older eastern islands with successive colonizations of more westerly islands in an east-to-west direction. However, long-distance dispersals among islands and extinction might have obscured the direction of past dispersal routes, generating other intricate patterns of colonization (Kim et al., 1996; Francisco-Ortega et al., 1996).

The aim of this study was to investigate the colonization and speciation patterns among recent Pliocene colonists of the Macaronesia region through (i) tests of independent versus related origin hypotheses, and (ii) tests of isolation by distance (IBD) and dispersal hypotheses. More than a decade of research has produced a substantial number of evolutionary studies of Macaronesian plants (see Carine et al., 2004, for a review). However, most correspond to presumably older Tertiary groups and have been based on a limited sample of sequences accessions. We were interested in testing whether the Macaronesian red fescues resulted from a single colonization, with subsequent, among-archipelago dispersal or resulted from multiple insular colonization events. The group is distributed in three out the four main archipelagos of Macaronesia (all except Cape Verde) and occupies an island land area of 450 km^2 embedded within a total spatial area of 450,000 km^2. The group therefore also serves as a model to investigate the potential rapid dispersal among Atlantic archipelagos separated by more than 800 km from each other. To our knowledge this is one of the first attempts to resolve the phylogeography of a recently radiated Macaronesian angiosperm plant group based on a large population sampling.

Analyses of AFLP markers are used to detect phylogeographical patterns among species and populations working at the interface of population differentiation and speciation. This technique has been successfully employed for resolving phylogenetic relationships in plant groups with low plastid and nuclear sequence variability (Koopman et al., 2001; Ogden and Thorpe, 2002; Despres et al., 2003; Koopman, 2005; Pimentel et al. 2007) and for characterizing the genetic diversity among individuals, populations, and species (Bensch and Åkesson, 2005; Althoff et al. 2007). Koopman (2005) concluded that AFLP markers produced reliable phylogenetic information when nuclear sequences are too conserved and are especially useful in the range of 0.016% to 0.05% sequence divergence, as observed in these otherwise unresolved Macaronesian red fescues (e.g., ITS: Catalán, 2006; Inda et al., 2008). In contrast to the patterns of rich species radiation observed in other Macaronesian endemic plant groups (e.g., *Argyranthemum*: Francisco-Ortega et al., 1996; *Echium*: Böhle et al., 1996; *Sonchus*: Kim et al., 1996; *Aeonium*: Jorgensen and Frydenberg, 1999; *Crambe*: Francisco-Ortega et al., 2002), the Macaronesian red fescues comprise four morphologically similar species that are almost archipelago specific. This could

facilitate the testing of IBD and colonization hypotheses from mainland ancestors that might have been affected by glaciations and the dynamic volcanic environment within old and newly arising archipelagos.

MATERIALS AND METHODS
Sample Collection and AFLP Analysis

We analyzed 36 populations of the four species of Macaronesian red *Festuca*: *F. agustinii* (15 populations), *F. jubata* (7 populations), *F. francoi* (7 populations), and *F. petraea* (7 populations). Sampling covered the geographical distribution of each species, totaling 215 individuals (Table 1, Fig. 1).

DNA was isolated following a modified CTAB protocol (Doyle and Doyle, 1987). The extraction DNeasy Plant Mini Kit of Qiagen was employed for small quantities of sample tissue in some cases. The concentration of each DNA sample was checked on 1% (0.5 × TBE) agarose gel using samples of known concentration. Approximately 200 ng of DNA was used for AFLP analysis following the instructions of Invitrogen manufacturers with slight modifications. *Eco*RI and *Mse*I restriction enzymes and their respective double-stranded adaptors were employed in three successive steps: digestion at 37°C for 2 h, heat inactivation of restriction enzymes at 70°C for 15 min, and ligation at 20°C for 2 h. Preselective amplification was performed after diluting the ligated DNA 10-fold with *Eco*RI+A and *Mse*I+C primers. PCR products were diluted 33-fold and used for selective amplification with *Eco*RI and *Mse*I primers plus three additional selective nucleotides. Seven combinations of *Eco*RI-*Mse*I primers were evaluated with two individuals of each of the four species. Two combinations (M-CAA/E-ACC and M-CAG/E-AAG) that provided a higher number of polymorphic markers were selected and used for the analysis of all samples. Amplifications were performed in PCT-100 MJ Research, Inc., and GeneAmp System 9700 Applied Biosystem thermocyclers. Preheated (50°C to 55°C) 6% polyacrylamide gels were run in 5× TBE electrophoresis buffer at 80 W for 2$^1/_2$ h. Gels were subjected to silver staining for visualization of bands following Bassam et al. (1991).

Accuracy of the AFLP markers was tested by reconducting the whole AFLP protocol in one individual per population in the most representative populations of each species (approximately 5% of the total sample size) and checking for consistency of recorded bands. As the calculated error rate (4.83%), which corresponded to the number of phenotypic differences related to the total number of phenotypic comparisons, was below the critical bound of 5% indicated in previous reports (Bonin et al., 2004, 2007; Pompanon et al., 2005; Piñeiro et al., 2007), the obtained AFLP patterns were considered to be highly reproducible. In order to increase the quality of the data, potentially unreliable bands that showed slight size differences among putative homologous bands across individuals, low-intensity bands, and either high (i.e., >410 bp) or low (i.e., <50 bp) molecular weights were discarded from the final data matrix.

TABLE 1. Data on sampled localities and AFLP variability of the four species of Macaronesian red *Festuca*: population codes, island location, number of individuals (N), number of unique fragments (UFr), and number of shared fragments among species. *agus = F. agustinii; juba = F. jubata; juba* = F. jubata* Fjuba6; *fran = F. francoi; petr = F. petraea*. Letters represent shared bands observed in at least two individuals per species. Total number of shared bands between a single pair of species is indicated in parentheses.

Population	Location	N	UFr	*agus fran* (12)	*agus juba* (3)	*agus petr* (2)	*juba fran* (2)	*juba petr* (2)	*fran petr* (1)
F. agustinii	Canary Islands	126	(7)						
Fagus1	Gran Canaria	3		ad	j				
Fagus2	Gran Canaria	5		abg	j				
Fagus3	Tenerife	10		adefg	hj	k			
Fagus4	Tenerife	6		adeg	ij	k			
Fagus5	Tenerife	12		acefg	hij	k			
Fagus6	Tenerife	10		abdefg	hj	k			
Fagus7	Tenerife	10		aceg	hij	k			
Fagus8	La Gomera	7		ace	hij				
Fagus9	La Gomera	10		ae	j	k			
Fagus10	La Palma	3		ae	j	k			
Fagus11	La Palma	6		af*g	j				
Fagus12	La Palma	12	1	abdeg	hij	l			
Fagus13	La Palma	13		abceg	hj	kl			
Fagus14	La Palma	9	1	ae	hj	k			
Fagus15	El Hierro	10		abeg	hij				
F. jubata	Madeira	27	(3)						
Fjuba1	Central peaks	3			hij				
Fjuba2	Central peaks	5			hj				
Fjuba3	Central peaks	5			hj				
Fjuba4	Central peaks	4			hj				
Fjuba5	Central peaks	4			hj		m		
Fjuba6	Curral das Freiras	5	3		h				
Fjuba7	Bica da Cana	3			hj			o	
F. francoi	Azores	33	(1)						
Ffran1	São Miguel	5		abce					
Ffran2	São Miguel	5		e			m		n
Ffran3	Terceira	4		be					n
Ffran4	Faial	4		bcdefg					n
Ffran5	Pico	5		defg					
Ffran6	São Jorge	5		befg					n
Ffran7	Flores	5					m		n
F. petraea	Azores	27	(1)						
Fpetr1	Santa María	4							
Fpetr2	Graciosa	3				k			n
Fpetr3	Graciosa	3				kl			n
Fpetr4	Faial	4				k			n
Fpetr5	Pico	5				k		o	n
Fpetr6	São Jorge	4				kl		o	n
Fpetr7	Flores	4							n

Interspecific Genetic Structure and Phylogenetic Analyses

The analyses performed were based on the assumptions that (i) despite the fact that AFLP markers behave as dominant markers, they could be used in simulated genotype-based analyses of diploid species such as the Macaronesian red fescues; (ii) comigrating fragments are considered homologous loci; (iii) genetic distances between heterozygous and homozygous individuals for specific loci are compensated across all surveyed loci; and (iv) even if loci might not all reconstruct the same coalescent history, the predominant sharing of homologous fragments indicate a common ancestry. Despite the problems associated with some of these assumptions, and given the high number of markers randomly gener-

ated from the whole genome, AFLPs has demonstrated to be a suitable technique to recover phylogeographic signal among closely related taxa and populations (Despres et al., 2003; Koopman, 2005; Pimentel et al., 2007; Althoff et al., 2007).

Bayesian analyses were conducted at the interspecific level, trying to estimate the genetic structure of taxa as a preliminary step for further testing of evolutionary speciation and colonization hypotheses. First, genetic structure was quantified at the species level according to the unbiased Bayesian-derived estimate G_{st}^{β}, related to Wright's F_{st} coefficient, based on the fixed-effect models proposed by Nei and Chesser (1983) using the program Hickory v.1.0.4 (Holsinger et al., 2002). Hickory's default

values (burn-in set to 50,000, sampling set to 250,000, and thin set to 50) were used to specify the prior distributions. AFLP data were analysed assuming two models: (1) free model and (2) $\theta^\beta = 0$ model, where θ^β is the among-populations fixation index. The deviance information criterion (DIC) was used to choose the model that best fitted to the data, based on their lower DIC values (Holsinger and Wallace 2004). A lower DIC value of the free model than the $\theta^\beta = 0$ model would be indicative of the existence of genetic structure in the data.

Second, to infer the spatial pattern of genetic divergence of the Macaronesian red *Festuca*, Bayesian model-based analysis was performed with STRUCTURE v.2.2 (Pritchard et al., 2000; Pritchard and Wen, 2004; Falush et al., 2007). The F model with admixed ancestry was used to estimate posterior probabilities of any predefined number (K) of groups (hereafter Bayesian groups), and individual percentages of membership were assigned to them according to their AFLP multilocus profiles (Falush et al., 2003, 2007). As a first strategy, a burn-in and a Monte Carlo Markov chain (MCMC) of 5000 and 20,000 iterations, respectively, were used in all these searches. Interspecific groupings were analyzed for different K groups of 50 runs each, analyzing simultaneously the studied populations of the Macaronesian red *Festuca*. A second strategy, involving 5×10^5 burn-in, 3×10^6 MCMC iterations, and 5 repetitions for K = 1–7 did not show any convergence into a unique clustering mode, so the first strategy was chosen to obtain reliable estimates for computing ΔK (rate of change in the log probability of data between successive K values; Evanno et al., 2005). The choice of the best K values was based on the following criteria: (i) selection of the higher ΔK; and (ii) the higher probability value ($\ln P(D)$; Pritchard and Wen, 2004) and the stability of clustering schemes (measured by a similarity coefficient [SC]) between different runs (Rosenberg et al., 2002). SC of >0.85 was taken as a measure of high stability. This coefficient was computed with the Matlab software (The Mathworks, 1994). We also considered the inference of a common individual membership (the α parameter) for the ancestral groups to compare it to α from non-structured groups (~1/K; Pritchard and Wen, 2004). Small α values imply that most individuals derive essentially from one or another ancestral Bayesian group, whereas $\alpha > 1$ values imply that most individuals are admixed (Pritchard and Wen, 2004).

Third, unrooted evolutionary relationships among the studied Macaronesian red *Festuca* samples were reconstructed based on a Bayesian approach to model AFLP marker evolution by nucleotide substitution and MCMC simulations (Luo et al., 2007). The genetic model assumes that a band might be lost due to mutations in the adaptor + restriction enzyme recognition sites or by a gain of a restriction site in the intermediate region. It was also assumed that all sites evolved independently with the same rate according to a Jukes-Cantor model. A total of 1.3×10^6 generations were simulated using simultaneously five parallel chains. Thirteen thousand trees were sampled discarding the first 800 trees per parallel chain to ensure convergence, totaling 9000 final trees for analysis. Preburn cycles were set to 10,000, tuning interval to 500, alphaA-tune = 10, alphaS-tune = 0.625, lambdaL-tune = 0.05, and lambdaG-tune = 0.05. All simulations were done with the software aflp v.1.01, kindly provided by R. Luo. The posterior distribution of trees was summarized through the posterior probabilities of common clades to all trees obtained from the aflp v.1.0.1 output. These probabilities were used to generate a priority consensus tree (PCT) in which compatible common clades were successively added to the tree according to their decreasing order of probability. Clades with probabilities less than 0.15 were treated as polytomies. The final Newick string of the PCT was edited with the *Dendroscope* tree viewer (Huson et al., 2007)

Species Colonization and Speciation Hypotheses

Due to the lack of any reliable sister group root that could provide a clearer direction of colonization and dispersal routes of the Macaronesian red fescues, and in order to infer the probability of shared history among species, the correlation structure of standardized residuals between species pairs were obtained according to Nicholson et al. (2002). Briefly, for each of the Bayesian groups defined according to clustering and phylogenetic analyses (i.e., *F. agustinii*, *F. jubata*, *F. francoi*, *F. petraea*) and each of the studied loci, the residuals were calculated by comparing estimated allele frequencies with those of the hypothetical ancestral population. Allele frequencies of the ancestor were obtained from the highest probability run out of 50 runs (best value for K = 4) using the no-admixture model with correlated allele frequencies.

The testing of the colonization and speciation history of the Macaronesian red fescues was done under the null hypothesis of pairwise independent colonization events from continental ancestors, each of them rendering a distinct species. This hypothesis was based on the unresolved basal placements of the Canarian, Madeiran, and Azorean red *Festuca* lineages (*Festuca* sect. *Aulaxyper* clade) obtained in the Loliinae phylogenies of Catalán (2006) and Inda et al. (2008). In this case, the absence of correlated residuals would suggest independent evolution between the compared species, whereas a negative or a positive correlation would suggest divergent or shared evolution, respectively. Shared evolution is compatible with a single origin from a continental source followed by interarchipelago dispersals and subsequent speciations.

Infraspecific Genetic Structure and Phylogenetic Analyses

Similar approaches to those previously assayed at the species level were undertaken at infraspecific level, to determine the genetic divergence and phylogenetic patterns of populations within species. Establishment of specific colonization hypothesis was preceded by (i) the detection of genetic homogeneous groups by mean of Bayesian STRUCTURE analysis, and (ii) the

reconstruction of rooted phylogenetic relationships among them using the infraspecific topologies of the PCT.

In order to establish infraspecific groups, STRUCTURE analysis for different K values were tested for each species, ranging from 1 to the number of sampled geographical populations plus two. At least 10 independent runs were computed for each K to adjust better the number of hypothetical groups within each species. A burn-in and an MCMC of 10^5 and 10^6 iterations were performed, respectively. Substructuring of *F. agustinii* K = 2 groups was based on 25 repetitions for each K with a burn-in and MCMC of 5×10^3 and 10^4 iterations, respectively. The choice of the best K values was based on previous interspecific criteria.

Infraspecific dispersal routes were generated according to the genetic composition of STRUCTURE Bayesian groups. It was assumed that populations within a Bayesian group were highly related to each other and consequently, infragroup population connections were favored over intergroup population connections. The root and order of colonization of the dispersal route with the highest correlation values (see next section) were established superimposing K = 2 Bayesian groups for *F. agustinii* and K = 3 Bayesian groups for *F. francoi* and *F. petraea* on the PCT. All individuals in a PCT clade containing a Bayesian group were then treated as a single genetic unit. In addition, given that it was of particular interest to evaluate the contribution of the easternmost Canarian *F. agustinii* individuals as a potential starting point of colonization, the eastern Bayesian group was further subdivided in the PCT into a Tenerife genetic unit and Gran Canaria genetic unit (see Results and Table 2). External nodes (i.e., those related to a Bayesian genetic unit) were located in the geographic vicinity of its individuals, whereas the geographic locations of the internal nodes were allowed to vary among different islands according to alternative dispersal hypotheses (see Table 3).

Infraspecific IBD and Dispersal Analysis

The strength of alternative colonization hypotheses for each of the four species was tested through simple and partial correlations between a matrix of genetic distance values and matrices of geographic or dispersal distance values among populations (Dietz, 1983; Smouse et al., 1986). Geographic distances are defined as the shortest linear distance between any given population pair where dispersion could have occurred in any possible direction (isolation by distance [IBD] model), whereas dispersal models include distances calculated as the sum of the linear distances connecting all the nodes between two populations for a given model (e.g., two or more steps). In some cases, an intermediate virtual node was created to connect geographically adjacent populations with other groups or populations. Population pairwise G_{st}^β estimates were calculated based on the free model of Hickory v.1.04 according to previously indicated interspecific parameters. Correlation analyses were computed with the program Phylogeographer v.1.1 (Buckler,

1999; Buckler et al., 2006). Significance was assessed by means of 10,000 permutations.

For each species, different dispersal routes were tested (see Figs. 3 to 5; Table 3), trying to identify the one that best explained the colonization patterns within each archipelago since the founding ancestor had speciated. We were interested to investigate whether the best colonization model followed an intuitive east-to-west dispersal pattern, concordant with the geological ages of the islands in each archipelago, or whether the optimal models did not respond to this general pattern. *Festuca jubata* was excluded from the analysis because in all tested models the correlation coefficients between genetic and dispersal distances (*r*) were not significantly different from zero.

RESULTS

Genetic Diversity and Structure of the Macaronesian Red Fescues

One hundred and eighty-one reliable polymorphic bands were generated across the four studied species using the two selective primers E-ACC/M-CAA and E-AAG/M-CAG. All individuals showed unique AFLP multilocus patterns. Unique and shared bands were observed at both species and population levels (Table 1). At the species level, *F. agustinii* showed the higher number of unique bands (7), followed by *F. jubata* (3) and *F. francoi* (1) and *F. petraea* (1). However, none of those bands served to characterize species as they tended to be in low frequencies. The number of exclusive shared bands between two species ranged from 1 (*F. francoi–F. petraea*) to 12 (*F. agustinii–F. francoi*), with a mean value of 3.6. Considering the whole set of polymorphic bands (181), the proportions of unique (range 0.56% to 3.87%) and shared (range 0.56% to 6.6%; mean 1.99%) bands were extremely low. At the population level, the Madeiran Fjuba6 population was the most diverse with three unique bands, whereas a single unique band was observed in the Canarian populations Fagus12 and Fagus14 (Table 1).

Free-model Bayesian analyses consistently gave lower DIC scores than $\theta^\beta = 0$ models, clearly supporting the existence of genetic structure among the studied *Festuca*; consequently, only free-model estimates of G_{st}^β are discussed. According to G_{st}^β, 31.0% of the overall variation within the Macaronesian red *Festuca* was attributable to differences among species; 26% of the total variation within *Festuca* was partitioned among archipelagos. G_{st}^β analysis revealed that 22.2% of the overall genetic variation contained in what was previously considered to be *F. jubata* sensu lato (*F. jubata + F. francoi*) was due to differences between the two species (see Discussion), whereas 15% was due to differences between the Azorean (*F. francoi, F. petraea*) species.

Interspecific Genetic Structure and Phylogenetic Analysis

The Bayesian priority consensus tree (PCT) recovered a high genetic structure among the Macaronesian red fescues (Fig. 2a, b). Almost all conspecific individuals

TABLE 2. Results from Bayesian-model clustering analysis of the Macaronesian red fescues conducted with STRUCTURE and Matlab software. K indicates the number of predefined Bayesian groups. ΔK is the second-order rate of change of the likelihood distribution (Evanno et al., 2005). Max ln$P$(X/K) and ln$P$(X/K) indicate the highest and the mean probability run values, respectively, for each K. SC is the mean similarity coefficient between all pairs of runs (Rosenberg et al., 2002). α is the admixture proportion of an individual and N(q$_K$) represents the number of individuals and the mean proportion (in brackets) of membership of all individuals associated with each inferred Bayesian group in the highest probability run. An individual was assigned to an inferred group according to its highest proportion of membership. Ø represents an empty group.

K	ΔK	Max ln$P$(X/K)	ln$P$(X/K)	SC	α	N(q$_K$)
All *Festuca* (*F. agustinii* + *F. Francoi* + *F. petraea* + *F. jubata*)						
1	—	−24,527.4	−24,568.1	—	—	
2	103.4	−20,963.5	−21,007.2	0.99	0.03	89(0.99); 126(0.99)
3	1.8	−19,563.1	−19,805.8	0.59	0.03	126(0.99); 60(0.98); 29(0.92)
4	0.1	−18,880.1	−19,205.4	0.38	0.03	126(0.99); 33(0.98); 27(0.97); 29(0.94)
5–7	0.3–1.8			0.18–0.32		
F. francoi+*F. petraea* + *F. jubata*						
1	—	−9,636.1	−9,662.4	—	—	
2	80.4	−8,260.3	−8,274.9	0.99	0.05	63(0.97); 26(0.96)
3	8.4	−7,581,2	−7,615.6	0.79	0.04	33(0.99); 27(0.97); 29(0.94)
4	0.3	−7,023.8	−7,390.9	0.90	0.03	5(0.99); 24(0.99); 33(0.99); 27(0.98)
5–6	7.0			0.45–0.48		
F. agustinii						
1	—	−11,184,6	−11,199.5	—	—	
2	195.7	−10,616.0	−10,618.5	0.99	0.07	66(0.97); 60(0.94)
3	2.2	−10,341.1	−10,347.3	0.99	0.09	48(0.89); 30(0.87); 48(0.86)
4	0.9	−10,026.2	−10,064.7	0.48	0.06	12(0.92); 32(0.92); 31(0.86); 51(0.86)
5	1.2	−9,772.9	−9,824,4	0.41	0.05	12(0.93); 32(0.93); 30(0.90); 24(0.87); 28(0.80)
6–17	0.1–8.6			0.00–0.37		
Eastern group						
2	—	−5,301.8	−5,352.2	0.59	0.23	38(0.88); 28(0.86)
3	—	−5,087.6	−5,123.1	0.73	0.09	22(0.88); 32(0.86); 12(0.82)
Western group						
2	—	−4,360.6	−4,372.8	0.66	0.04	45(0.99); 12(0.98)
3	—	−4,099.1	−4,159.3	0.57	0.04	12(0.97); 38(0.97); 7(0.93)
F. francoi						
1	—	−3,054.3	−3,065.1	—	—	
2	18.1	−2,856.6	−2,862.9	0.64	0.06	10(0.98); 23(0.98)
3	123.7	−2,556.3	−2,559.2	1.00	0.05	18(0.97); 5(0.96); 10(0.93)
4	4.2	−2,418.3	−2,440.1	0.68	0.05	5(0.95); 13(0.94); 5(0.93); 10(0.93)
5	13.7	−2,325.8	−2,480.7	0.65	0.08	5(0.94); 5(0.91); 10(0.9); 9(0.81); 4(0.78)
6	3.3	−2,918.4	−4,251.1	0.64	0.04	Ø; Ø; 5(0.96); 5(0.94); 10(0.90); 13(0.89)
7–9	0.81			0.53–0.68		
F. petraea						
1	—	−1,963.6	−1,972.7	—	—	
2	17.4	−1,695.1	−1,701.4	0.84	0.12	13(0.95); 14(0.91)
3	1.3	−1,555.6	−1,609.2	0.76	0.05	4(0.99); 8(0.96); 15(0.91)
4	92.4	−1,423.1	−1,427.8	0.99	0.04	4(0.99); 4(0.99); 12(0.97); 7(0.85)
5	5.3	−1,461.3	−1,468.2	0.99	0.04	Ø; 4(0.99); 4(0.99); 12(0.97); 7(0.82)
6	0.5	−1,480.5	−1,482.8	0.99	0.03	Ø; Ø; 4(0.99); 4(0.98); 12(0.96); 7(0.80)
7–9	0.2–0.8			0.66–0.92		
F. jubata						
1	—	−2,381.4	−2,396.7	—	—	
2	290.6	−1,881.0	−1,886.5	1.0	0.03	5(1.00); 24(1.00);
3	1.2	−1,817.0	−3,365.3	0.66	0.04	5(0.99); 4(0.91); 20(0.89);
4	0.4	−1,709.0	−3,535.9	0.48	0.05	5(0.99); 11(0.92); 4(0.89); 9(0.83)
5	0.5	−1,659.9	−2,852.6	0.56	0.05	5(0.98); 4(0.90); 9(0.89); 2(0.88); 9(0.88)
6	0.7	−1,922.3	−3,062.6	0.75	0.03	Ø; Ø; Ø; Ø; 5(0.97); 24(0.97)
7–9	0.5–0.6			0.82–0.92		

were monophyletic; only *F. francoi* showed paraphyly with *F. petraea* apparently derived from within it. In general, species and geographic branches had weaker support (Azores: 0.24 posterior probability support [PS], *F. petraea*: 0.60 PS, *F. agustinii* 0.42 PS, and *F. jubata*: 0.33 PS) than terminal branches (Fig. 2).

The same trend of genetic divergence among the Macaronesian red *Festuca* was observed in the Bayesian analyses performed with STRUCTURE (Fig. 2, Table 2). Higher values of ΔK and SC and lower values of α indicated that K = 2 appropriately represents the num-

ber of optimal Bayesian groups for the four Macaronesian red fescues. For K > 2, increasingly higher values of ln$P$(D) were observed, indicating the existence of additional structure; however, lower ΔK and SC < 0.59 values also suggested the existence of a complex structure with many different groups that generated multiple solutions for the same K (Table 2). The most robust result (K = 2) clearly separated the Canarian *F. agustinii* from the remaining species (Fig. 2c1), suggesting that this species is the most divergent one, although sample sizes and genetic diversity could have influenced the splitting order

TABLE 3. Dispersion models analyzed in three Macaronesian red fescues (*F. agustinii, F. francoi, F. petraea*). The numbers represent abbreviations of population codes given in Table 1. All connective paths are read from left to right, beginning with the easternmost population(s) of each archipelago. Populations in parentheses were joined by a proximal geographic hypothetical node. Double slashes represent a principal bi- or trifurcation. Single slashes represent a secondary bi- or trifurcation. The best five dispersion models of each species have been represented in Figures 3 to 5.

Model	Connective path
F. agustinii	
Model 1	(1,2)//(1,2)-(8,9)//(1,2)-3/3-(4,6,7)-5/3-(10,11)-(12,13,14)-15
Model 2	(1,2)//(1,2)-9//(1,2)-3/3-(4,6,7)-5/3-(10,11)-(12,13,14)-15-8
Model 3	(1,2)-3//3-(4,6,7)-5-(8,9)//3-(10,11)-(12,13,14)-15
Model 4	(1,2)-3//3-(4,6,7)-5-9/3-(10,11)-(12,13,14)-15-8
Model 5	(1,2)-3//3-(4,6,7)-5-9//3-(10,11)-(12,13,14)/(12,13,14)-15/(12,13,14)-8
Model 6	(1,2)-(4,6,7)//(4,6,7)-5-(8,9)//(4,6,7)-3-(10,11)-(12,13,14)-15
Model 7	(1,2)-(4,6,7)//(4,6,7)-5-9//(4,6,7)-3-(10,11)-(12,13,14)/(12,13,14)-15/(12,13,14)-8
Model 8	(1,2)-5//5-(8,9)//5-(4,6,7)-3-(10,11)-(12,13,14)-15
Model 9	(1,2)-5//5-9//5-(4,6,7)-3-(10,11)-(12,13,14)/(12,13,14)-15/(12,13,14)-8
Model 10	(1,2)//(1,2)-9//(1,2)-3-(4,6,7)-5//(1,2)-(10,11)-(12,13,14)-15-8
Model 11	(1,2)//(1,2)-3-(4,6,7)-5-9//(1,2)-(10,11)-(12,13,14)-15-8
Model 12	(1,2)-3//3-(10,11)//3-(4,6,7)/(4,6,7)-(12,13,14)-15/(4,6,7)-5-(8,9)
Model 13	(1,2)-(4,6,7)//(4,6,7)-(8,9)//(4,6,7)-(12,13,14)-15//(4,6,7)-3-(10,11)
Model 14	(1,2)-(8,9)-5-(4,6,7)-3-(10,11)-(12,13,14)-15
Model 15	(1,2)-3-(4,6,7)-5-9-8-(10,11)-(12,13,14)-15
Model 16	(1,2)-5//5-(4,6,7)-3//5-9-8-(10,11)-(12,13,14)-15
Model 17	(1,2)//(1,2)-3-(4,6,7)-5//(1,2)-9-(10,11)-(12,13,14)/(12,13,14)-15/(12,13,14)-8
Model 18	(1,2)-3//3-(4,6,7)-5-9-8-(12,13,14)-15//3-(10,11)
Model 19	(1,2)-(4,6,7)//(4,6,7)-3-(10,11)//(4,6,7)-5-9-8-(12,13,14)-15
Model 20	(1,2)-(10,11)//(10,11)-(12,13,14)-15-8//(10,11)-3-(4,6,7)-5-9
Model 21	(1,2)//(1,2)-9//(1,2)-(10,11)/(10,11)-(12,13,14)-15-8/(10,11)-3-(4,6,7)-5
F. francoi	
Model 1	(1,2)-3//3-5//3-6-4//3-7
Model 2	(1,2)//(1,2)-7//(1,2)-3/3-5/3-6-4
Model 3	(1,2)-3//3-6-5//3-4//3-7
Model 4	(1,2)//(1,2)-7//(1,2)-3-6-5-4
Model 5	(1,2)-3//3-6-5-4//3-7
Model 6	(1,2)-3//3-6-5-7//3-4
Model 7	(1,2)-3-6-5//5-4//5-7
Model 8	(1,2)-4//4-7//4-6/6-5/6-3
Model 9	(1,2)-5//5-7//5-4//5-6-3
Model 10	(1,2)-4//4-7//4-5//4-6-3
Model 11	(1,2)//(1,2)-4//(1,2)-3/3-6-5/3-7
Model 12	(1,2)//(1,2)-6-5-7//(1,2)-3-4
Model 13	(1,2)//(1,2)-4//(1,2)-3-6-5-7
Model 14	(1,2)-7-3//3-5//3-6-4
Model 15	(1,2)-7//7-3//7-(4,5,6)
F. petraea	
Model 1	1-4//4-2-3-6-5//4-7
Model 2	1-(2,3)//(2,3)-4//(2,3)-6-5//(2,3)-7
Model 3	1-(2,3)//(2,3)-4-7//(2,3)-6-5
Model 4	1-(2,4)-(3,5,6)-7
Model 5	1-(2,4)-7-3-6-5
Model 6	1//1-7//1-4-2-3-6-5
Model 7	1//1-7//1-(2,3)/(2,3)-6-5/(2,3)-4
Model 8	1//1-7//1-2-4//1-3-6-5
Model 9	1//1-3-6-5//1-2/2-4/2-7
Model 10	1//1-3-6-5//1-2-4-7
Model 11	1//1-3-6-5//1-4/4-2/4-7
Model 12	1//1-2-4//1-3/3-6-5/3-7
Model 13	1//1-7-(3,5,6)//1-(2,4)

(Rosenberg et al., 2002). Nevertheless, this divergence should not be interpreted as indicative of ancestry, since STRUCTURE does not take into account any relationship of Bayesian groups to an evolutionary root.

Further substructuring was analyzed for the Bayesian group comprising *F. petraea* + *F. francoi* + *F. jubata*. Here, ΔK supported the existence of two groups (K = 2), formed by *F. jubata* and by *F. petraea* + *F. francoi*, although K = 3 could also represent the actual number of groups,

because this grouping also showed a relatively high SC = 0.79 and a higher probability than K = 2 (Table 2, Fig. 2c2). *Festuca jubata* was the most divergent species followed by the separation of *F. francoi* and *F. petraea* in the STRUC-TURE analyses (Fig. 2c2) and in the PCT (Fig. 2a).

Hypothesis Testing of Species' Origins

The no-admixture model of STRUCTURE for K = 4 was further assessed to infer the possibility of
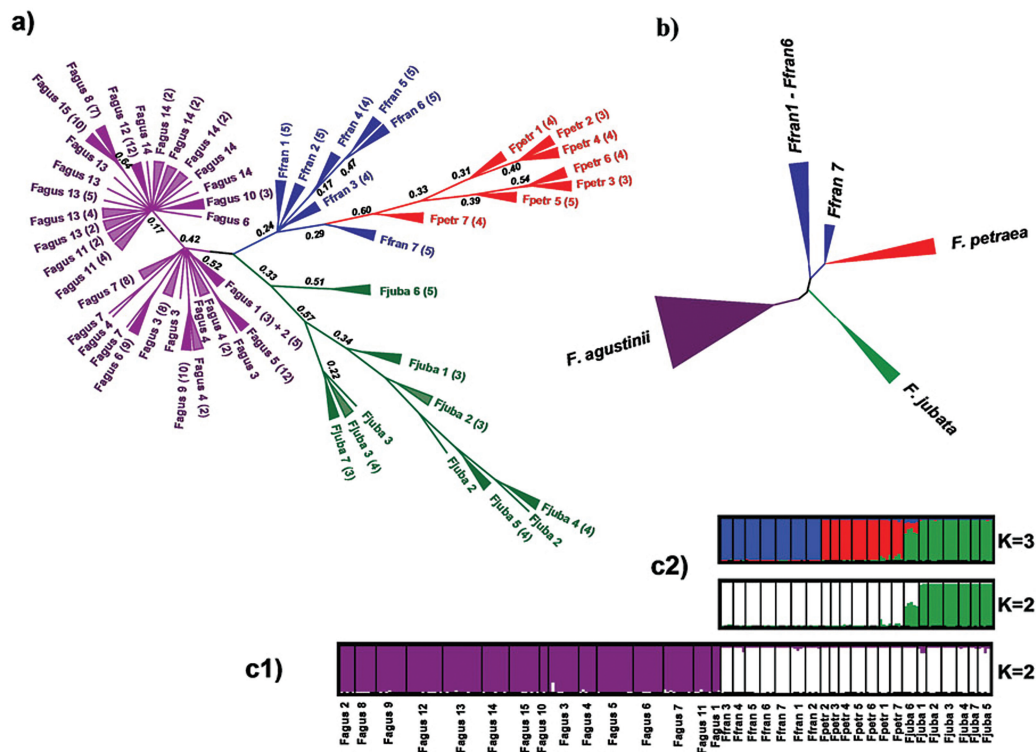
FIGURE 2. Supraspecific Bayesian phylogenetic and structure analyses of the four Macaronesian red fescues (*Festuca* sect. *Aulaxyper*): *F. agustinii* (purple); *F. jubata* (dark green); *F. francoi* (blue); *F. petraea* (red). (a) Priority consensus tree based on Bayesian MCMC strategy. Population codes correspond to those indicated in Table 1. Filled triangles represent collapsed whole populations; dotted triangles represent partial populations. Values on branches indicate posterior probabilities of clades. (b) PCT constructed as for Figure 2 but with all conspecific individuals represented by triangles. (c) Diagrams showing the proportion of membership of each individual to the inferred Bayesian groups: (c1) K = 2 for the total number of individuals; (c2) K = 2–3 with *F. agustinii* individuals excluded from the analysis (see comments in text). Gross vertical black bars separate populations, which are formed by thin vertical colored individual bars.

independent evolution of each of the four Macaronesian red fescues. The no-admixture model estimates the allelic frequencies of the putative ancestor of the analyzed species and those frequencies are subsequently used for the correlation analysis of the species' standardized residuals. This approach was used to investigate the null hypothesis of independent evolution of allele frequencies of each pair of the four Macaronesian red fescues.Exclusion of Fjuba6 from the analysis was based on previous results obtained with the admixture model analysis (Fig. 2c2) that showed that this population generated a non-discrete condition to *F. jubata*.

According to the correlation structure of standardized residuals among species, three sets of correlations are observed: (i) positive correlation between *F. francoi* and *F. petraea* ($r = 0.264$, $P < 0.01$); (ii) pairwise negative correlations between *F. agustinii* and *F. francoi* ($r = -0.381$, $P < 0.01$), *F. agustinii* and *F. petraea* ($r = -0.24$, $P < 0.01$), and *F. jubata* and *F. francoi* ($r = -0.196$, $P < 0.01$); and (iii) no-correlation between *F. jubata* and *F. agustinii* ($r = -0.01$, $P = 0.84$) and *F. jubata* and *F. petraea* ($r = -0.055$, $P = 0.47$). Shared evolutionary history is only suggested for *F. petraea* and *F. francoi*. The remaining comparisons indicate a clear divergent evolutionary history between *F. agustinii* and the two Azorean species, despite the rela-

tively high number of shared fragments between *F. agustinii* and *F. francoi* (Table 1), and an independent evolutionary pattern between *F. jubata* and the remaining taxa. These results are compatible with a single colonization event to Azores and two independent colonization events to Madeira and the Canary Islands, respectively.

Spatial Structure and Phylogeographic Patterns of Populations

The PCT (Fig. 2a) detected a high genetic structure among 25 out of the 36 analyzed populations (69.4% of the total). This included all populations of *F. francoi*, and *F. petraea*, 5 of *F. jubata*, and 6 of *F. agustinii* that showed all their individuals clustered monophyletically with moderate to low posterior probabilities.

At the species level, higher values of SC, ln$P$(D), and ΔK were observed for K < 5 (Table 2). Also, some empty groups were obtained for K = 5 or K = 6 for *F. petraea*, *F. francoi*, and *F. jubata*. Consequently, only Bayesian groups of up to K = 4 were taken into account for phylogeographical analysis. ΔK values supported 2 groups for *F. agustinii* and *F. jubata*, 3 for *F. francoi*, and 4 for *F. petraea* (Table 2).

Festuca agustinii. At K = 2, two Bayesian groups and one putatively admixed Fagus10 population (Fig. 3a)
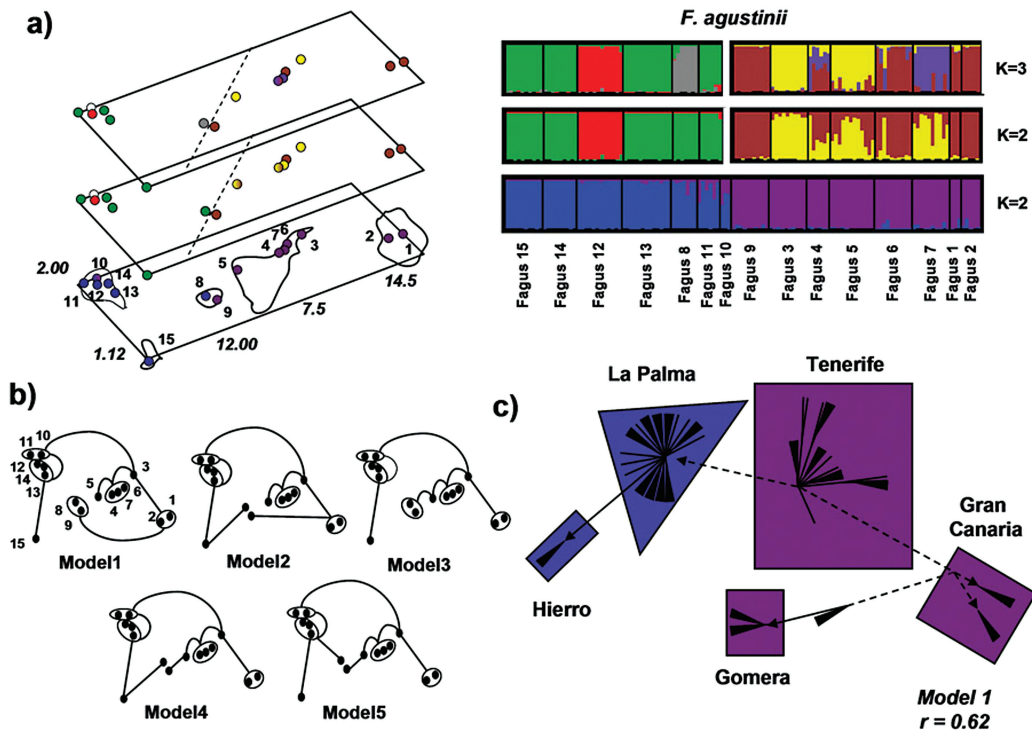
FIGURE 3. (a) Model-based Bayesian analysis of *Festuca agustinii*. For each K the vertical black bars of the histogram separate different populations and their colors represent the proportion of individual membership to each inferred Bayesian group. Each Bayesian analysis is accompanied by a superposition of geographical maps (each for a different K) where populations are located. Dots represent populations and their color is related to the Bayesian group inferred at the respective K. In italics, the geological age of each island in Ma (million years ago). (b) Models or dispersion routes of *Festuca agustinii* in the Canary Islands. Connective lines represent dispersion paths. Ovals frame populations connected to a proximal ancestor geographic node. Only the five dispersal models with higher correlation with genetic distance are shown (see Table 4). (c) Superposition of the Bayesian structure groups over the *F. agustinii* partial priority consensus tree. *r* values indicate the correlation coefficient of dispersal model 1 matrix with G_{st}^{β} genetic distance matrix.

were inferred, respectively, in this species. In this sense, Fagus10 emerges as a possible genetic bridge between the two Bayesian groups. One group was formed by the eastern Gran Canaria, Tenerife, and Fagus9 (La Gomera) populations and another group by the western La Palma, El Hierro, and Fagus8 (La Gomera) populations. The western group was resolved as monophyletic in the PCT (Fig. 2a). Within this group, it was also observed a monophyletic group formed by Fagus15 (El Hierro) and Fagus8 (La Gomera) populations. Substructuring revealed that the eastern group was further subdivided into a southern Gran Canaria + Gomera group and a Tenerife group (K = 2; Fig. 3a) and that the central populations of Tenerife are differentiated from the northern and southern ones (K=3; Fig. 3a), with Tenerife Fagus6 showing more genetic similarity to Gran Canaria than to other Tenerife populations.

Festuca jubata. At K = 2, individuals of the two inferred Bayesian groups showed full percentage of membership to their respective groups. One group included population Fjuba6, which diverged first from the clade of the remaining *F. jubata* populations in the PCT (Fig. 2a). The later group was further subdivided at K = 3 and K = 4 but without resulting in a geographic arrangement. The large geographic distance (12 km) that separates population Fjuba7 from populations Fjuba1 to Fjuba5 (Fig. 1)

was not paralleled by any genetic divergence between those groups of conspecific individuals.

Festuca francoi. At K = 2, an eastern group formed by São Miguel populations (Ffran1, Ffran2) was separated from the remaining ones, located in the central and western subarchipelagos (Fig. 4a). The inferred Bayesian groups were represented by individuals with high proportions of membership to their respective clusters. At K = 3, the central-western group was subdivided into the western (Ffran7, Flores) and central groups. The K = 4 model separated Ffran5 (Pico) from the rest of the central subgroup. The PCT did not show any basal resolution to define whether the eastern and western subarchipelago groups are sister or paraphyletic clades. (Fig. 2a).

Festuca petraea. At K = 2, the Bayesian groups did not show geographical structure as in one of those groups there was a close connection of populations Fpetr1 (Santa Maria), Fpetr4 (Faial), and Fpetr7 (Flores), located in the eastern, central, and western subarchipelagos, respectively (Fig. 5a). That group was further resolved as a cline at K = 3 and K = 4, suggesting a progressive genetic differentiation of populations in a southeastern-northwestern direction. The second group was formed exclusively by central populations (Fpetr3, Graciosa; Fpetr5, Pico; Fpetr6, São Jorge) and was not further subdivided at K = 3 to 4. Fpetr2 (Graciosa) showed an
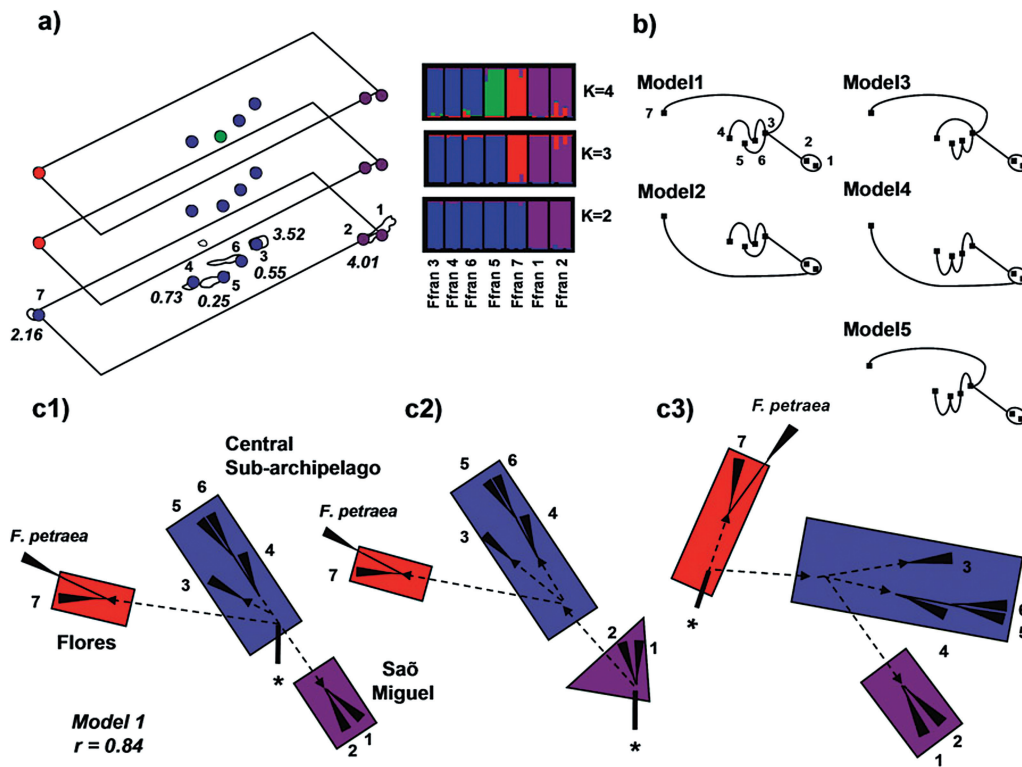
FIGURE 4. (a) Model-based Bayesian analysis of *Festuca francoi*. For each K the vertical black bars of the histogram separate different popula-tions and their colors represent the proportion of individual membership to each inferred Bayesian group. Each Bayesian analysis is accompanied by a superposition of geographical maps (each for a different K) where populations are located. Dots represent populations and their color is related to the Bayesian group inferred at the respective K. In italics, the geological age of each island in Ma (million years ago). (b) Models or dispersion routes of *Festuca francoi* in the Azores archipelago. Connective lines represent dispersion paths. Ovals frame populations connected to a proximal ancestor geographic node. Only the five dispersal models with higher correlation with genetic distance are shown (see Table 4). (c) Superposition of the Bayesian structure groups over the *F. francoi* partial priority consensus tree. (c1–c3) Three different placements of the root (with asterisk) compatible with model 1 dispersal route. *r* values indicates the correlation coefficient of dispersal model 1 matrix with G_{st}^{β} genetic distance matrix.

admixed constitution, probably connecting Fpetr4 with other members of the central subarchipelago group. The PCT indicated that the first group was paraphyletic with respect to the central subarchipelago group (Fig. 2a).

Hypothesis Testing of Populations' IBD and Dispersal Models

Dispersal models were tested at the population level for the Macaronesian red fescues. Different hypothetical dispersal routes of populations, deduced from the diver-gent Bayesian groups obtained from STRUCTURE and their potential geographical connections, were assayed for *F. agustinii* (21), *F. francoi* (15), and *F. petraea* (13) (Ta-ble 3). Populations of *F. jubata* were excluded from this analysis, given that none of the dispersal routes tested resulted in significant correlations.

The five routes that showed the highest simple (*r*) and multiple (R^2) correlation coefficients between genetic and dispersal distances (dispersal models) as well as be-tween genetic and geographic distances (IBD model) are shown in Figures 3 to 5 and Table 4. Model 1 showed a higher correlation value than the alternative models in all cases (Table 4). This dispersal model also had a higher

correlation value than the IBD model. Partial correlations of model 1|IBD model indicated that the dispersal dis-tance model 1 explained a significant part of the variation that was not explained by the IBD model, whereas par-tial correlations of IBD model | model 1 were nonsignif-icant, indicating that all the variation explained by the IBD model was already explained by the dispersal dis-tance model 1. Also, the differences observed between the simple coefficient of determination and the coeffi-cient of multiple determination indicated the relative merit of adding another source of variation once the first source was fitted. Model 1 and the multiple coefficient of determination were similar to each other, whereas the IBD model was lower than the multiple coefficient, im-plying that substantial information was gained adding model 1 once the IBD model was fitted.

In order to clarify the most likely direction of the col-onization in model 1, Bayesian genetic units derived from the PCT were considered. Only one configuration is compatible with model 1 dispersal route for *F. agus-tinii,* whereas two are compatible for *F. petraea*. The PCT topology of *F. francoi* (plus *F. petraea*) clades (Fig. 2a) gen-erated three alternative dispersal model 1 configurations,
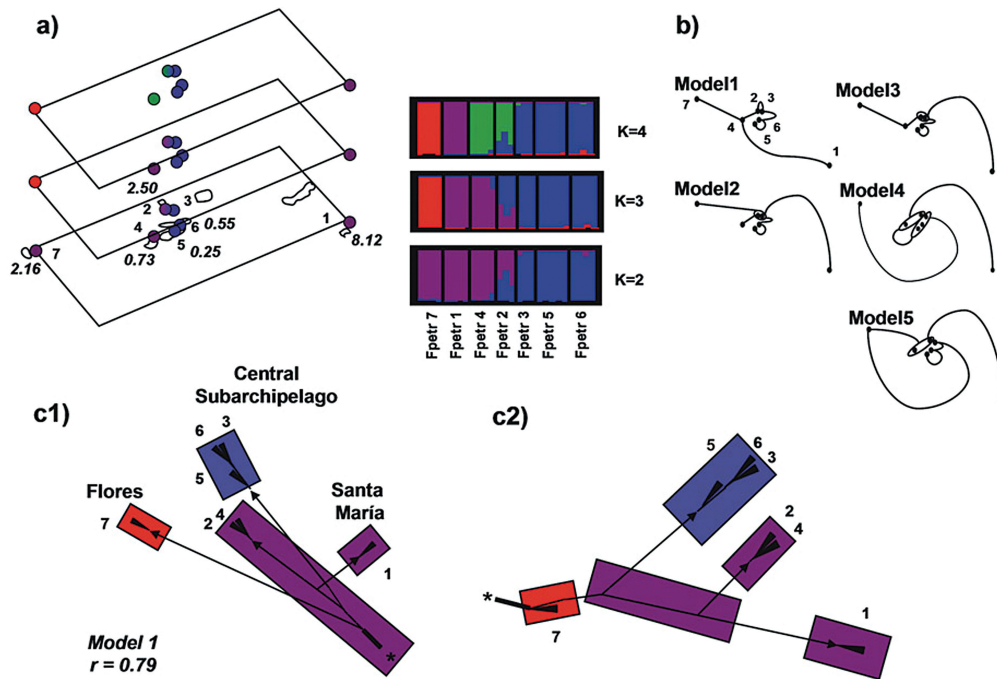
FIGURE 5. (a) Model-based Bayesian analysis of *Festuca petraea*. For each K the vertical black bars of the histogram separate different populations and their colors represent the proportion of individual membership to each inferred Bayesian group. Each Bayesian analysis is accompanied by a superposition of geographical maps (each for a different K) where populations are located. Dots represent populations and their color is related to the Bayesian group inferred at the respective K. In italics, the geological age of each island in Ma (million years ago). (b) Models or dispersion routes of *Festuca petraea* in the Azores archipelago. Connective lines represent dispersion paths. Ovals frame populations connected to a proximal ancestor geographic node. Only the five dispersal models with higher correlation with genetic distance are shown (see Table 4). (c) Superposition of the Bayesian structure groups over the *F. petraea* partial priority consensus tree. (c1, c2) Two different placements of the root (with asterisk) compatible with model 1 dispersal route. *r* values indicates the correlation coefficient of dispersal model 1 matrix with G_{st}^{β} genetic distance matrix.

which agreed with equal number of different geographic locations of the root node in each of the Bayesian genetic units.

Model 1 of *F. agustinii* (Fig. 3b, c) suggests that the colonization of the Canary Islands could have started from northern Tenerife following two bidirectional dispersal colonizations, one to the easternmost Gran Canaria island and central La Gomera island and the other to the western islands of La Palma, and El Hierro, plus a further parallel colonization of southern Tenerife. Sequential analysis of K = 2 to K = 3 Bayesian groups suggests that colonization of Gran Canaria and La Gomera must have been of relatively recent origin, as populations Fagus1 and Fagus2 (Gran Canaria) and populations Fagus8 and Fagus9 (La Gomera) are not further differentiated from populations Fagus4 or Fagus6 from Central Tenerife. Other potential configurations in model 1 are not supported by any of the best five correlation coefficient models (e.g., placing the root in Gran Canaria or La Palma genetic units would imply two independent colonizations to each of the two other genetic units, respectively). A further retrocolonization event suggested by STRUCTURE analysis and PCT, from the younger island of El Hierro to the older island of La Gomera (Fig. 3a), is supported by models 2, 4, and 5 (Fig. 3b).

F. francoi model 1 (Fig. 4b, c1, c2, c3) supports a stepping-stone colonization, involving the central

subarchipelago genetic unit as an intermediate step between the eastern and western subarchipelagos. Nevertheless, the root could not be placed unambiguously into a unique location, considering that the three genetic units were connected by one basal polytomy (Fig. 2a). This generated three different alternative hypotheses to explain model 1. Sequential analysis of K = 2 to K = 3 Bayesian groups (Fig. 4a) indicates that Ffran 7 population (Flores) could have diverged after the main split between eastern and the western + central subarchipelago populations. This rules out model 1c1 and 1c3 scenarios, adding support for the stepping-stone east-to-west colonization route of model 1c2 (Fig. 4c).

For *F. petraea*, model 1 (Fig. 5b, c1, c2, c3) suggests the same scenario as for *F. francoi*, involving a central subarchipelago intermediate colonization step. Two different model 1 configurations could explain, however, this colonization pattern (Fig. 5c1, c2). Scenario model 1c1 suggests that the colonization trend of *F. petraea* could have started in the central subarchipelago islands of Faial or Graciosa and that the eastern and western subarchipelagos would be colonized independently. Under such an assumption, the Fpetr3 + Fpetr5 + Fpetr6 Bayesian group would be of ancient origin, given its extreme divergence from the rest of populations in the K = 2 STRUCTURE analysis (Fig. 5a), although another alternative hypothesis would imply a recent divergence event

TABLE 4. Simple and partial correlation analyses between genetic G_{st}^{β} distances and geographic or dispersal distances for three Macaronesian red fescues (*F. agustinii, F. francoi, F. petraea*). Dispersion models correspond to those described in Figures 3 to 5 and Table 3; they correspond to the dispersal models that showed the highest correlation with genetic distance. r = simple correlation coefficient between genetic and geographic or dispersion model distances. P = probability for a random r higher than observed r after 10,000 permutations. $r_{M|G}$ = partial correlation coefficient between genetic and dispersal model distances once geographic distance was fixed. r^2 = coefficient of determination. R^2 = coefficient of multiple determination.

Model	r	**P**	r^2	$r_{M|G}$	**P**	$r_{G|M}$	**P**	R^2
F.agustinii								
Geographic	0.3399	0.0062	0.1156					
Model 1	0.6170	<0.001	0.3807	0.5493	<0.001	0.0531	0.4111	0.3824
Model 2	0.5717	<0.001	0.3268	0.4975	0.0051	0.1062	0.3015	0.3344
Model 3	0.5705	<0.001	0.3255	0.4978	<0.001	−0.1171	0.7997	0.3347
Model 4	0.5625	<0.001	0.3164	0.4957	<0.001	−0.1554	0.8723	0.3329
Model 5	0.5526	<0.001	0.3054	0.4658	<0.001	−0.0545	0.6670	0.3074
F. francoi								
Geographic	0.6989	0.0145	0.4884					
Model 1	0.8446	0.0010	0.7134	0.7030	0.0080	−0.3118	0.8593	0.7412
Model 2	0.8254	0.0013	0.6812	0.6518	0.0140	0.2775	0.1899	0.7058
Model 3	0.8172	0.0014	0.6678	0.6141	0.0273	−0.2019	0.7481	0.6814
Model 4	0.7925	0.0019	0.6280	0.5713	0.0238	0.2712	0.1822	0.6554
Model 5	0.7875	0.0044	0.6201	0.5317	0.0097	−0.1847	0.8090	0.6331
F. petraea								
Geographic	0.6900	0.0167	0.4761					
Model 1	0.7929	<0.001	0.6287	0.6184	0.0028	−0.3587	0.9364	0.6765
Model 2	0.7598	0.0054	0.5773	0.5156	0.0448	−0.3002	0.8201	0.6154
Model 3	0.7520	<0.001	0.5656	0.5023	0.0272	−0.3137	0.8689	0.6083
Model 4	0.5711	0.0329	0.3261	0.2434	0.2937	0.5183	0.0836	0.5072
Model 5	0.5388	0.0599	0.2903	0.2025	0.3446	0.5405	0.0708	0.4976

that would require extreme genetic changes during colonization. Scenario model 1c2 is compatible with a putative origin of *F. petraea* in Flores, which agrees with the close relationship between this species and the Flores *F. francoi* Ffran7 population in the PCT (Fig. 2a). In this case, the colonization would follow a west-to-east direction, opposite to that of *F. francoi*.

DISCUSSION

Origin and Diversification of the Macaronesian Red Fescues

In contrast to other endemic angiosperm groups that show high ecological adaptation and pronounced speciation in Macaronesia (e.g., *Argyranthemum*: Francisco-Ortega et al., 1996; *Echium*: Böhle et al., 1996; *Sonchus*: Kim et al., 1996; *Tolpis*: Moore et al., 2002, Archibald et al., 2006; and the Macaronesian Crassulaceae clade [i.e., *Aeonium, Aichryson,* and *Monanthes*]: Mort et al., 2002; Fairfield et al., 2004), there are only four endemic species of red fescues. However, these species are distributed in all Macaronesian archipelagos, with the exception of the Cape Verde, making them an ideal model to test dispersal models of plants with similar diversity rates in this large oceanic region of more than 450,000 km². Furthermore, the recent Pliocene origin of the Macaronesian red fescues has allowed us to test if alternative multiple colonization or in situ speciation episodes could have occurred during the short span of 2.5 ± 0.9 Ma time that has elapsed since they diverged from their common continental *Festuca* sect. *Aulaxyper* ancestor (Catalán, 2006; Inda et al., 2008).

The close, but unresolved, evolutionary relationships recovered for three (*F. agustinii, F. jubata,* the Azorean group) of the four lineages of Macaronesian red fescues in previous studies of subtribe Loliinae based on plastid and nuclear sequence data (Inda et al., 2008) are not inconsistent with at least three independent long-distance dispersal events from the continent to each separate archipelago (i.e., the Canaries, Madeira, and the Azores). Moreover, the strong sister group relationship recovered for the two Azorean species indicated that they probably evolved from a common ancestor that colonized the Azores during the Pleistocene (ca. 1.1 ± 0.6 Ma; cf. Inda et al., 2008). These hypotheses have been partially confirmed and further illuminated by the present study. The relatively high number of shared AFLP fragments among populations of different island species (Table 1) and the low number of unique, non-fixed fragments in species and populations suggest a recent origin of these species. The STRUCTURE and PCT analyses illustrate the greater divergence of the Canarian *F. agustinii* with respect to the remaining species and the separation of the Madeiran *F. jubata* from the Azorean group of the closely related *F. francoi* and *F. petraea* (Fig. 2a, b, c1, c2). The possession of the highest number of private fragments further highlights the genetic distinctness of *F. agustinii* from the others (Table 1). All these data support a more ancient isolation of *F. agustinii* in the Canary Islands and of *F. jubata* in Madeira and a more recent isolation of *F. francoi* and *F. petraea* in the Azores but do not allow inferences to be made about the mono- or polyphyletic origin of the group. Some drawbacks that could preclude the resolution of the origins of the Macaronesian red fescues relate to the present restricted distribution of the close relative *F. rivularis* in SW Europe and the possible extinction of other close ancestral diploid lineages in this region and in NW Africa after the colonization of the oceanic islands. This has also been hypothesized for

other angiosperms (*Tolpis*: Moore et al., 2002; *Androcymbium*: Caujapé-Castells, 2004).

The analysis of correlation of standardized residuals based on hypervariable AFLP data has allowed us to infer a possible evolutionary scenario related to the origin and divergence of these Atlantic oceanic grasses, although it should be considered a preliminary hypothesis, given that the use of correlations among residuals to detect deviations from the model of independent evolution (such as shared history or migration) has not been extensively evaluated (G. Nicholson, personal communication). Our results suggest three colonization events: one to the Canary Islands probably from northwest Africa, resulting in *F. agustinii*, a second one to Madeira, probably from southwest Europe, resulting in *F. jubata*, and a third one to Azores, probably from western Europe, resulting in the ancestor of *F. francoi* and *F. petraea*.

The priority consensus tree (PCT) suggests a possible scenario where *F. petraea* could have diverged from *F. francoi*, as indicated by the PCT node that locates Ffran 7 (Flores) as a sister population to *F. petraea*. Assumed evidence for the existence of breeding barriers between the two Azorean species (St-Yves, 1922; Dias, 2001) has been corroborated by our genetic data: (a) Bayesian results that clearly discriminated individuals of each species (Fig. 2); and (b) Bayesian G_{st}^{β} that give an estimate of 15%, indicative of a pronounced genetic differentiation (Hartl and Clark, 1997). Although multiple colonization events cannot be totally ruled out because westerly wind currents may facilitate the transport of diaspores to the Azores, the archipelago is an extremely difficult target for such events given that it has a small land area relative to the Atlantic Ocean and is isolated by at least 800 km from any source of colonization (Fig. 1). Adaptive speciation following a single colonization event is therefore consistent with the data and the most probable scenario to explain the existence of divergent sympatric gene pools in the archipelago (see Moore et al., 2002). The possibility of adaptive speciation is suggested by differences in morphology and ecological habitats. Thus, *F. petraea* and *F. francoi* are associated with coastal and with humid highland environments, respectively, with few contact zones except in the coastal cliffs of the westernmost islands of Flores and Corvo (Dias, 2001). Ecological speciation could have been prompted by geographic, stochastic, and genetic factors (Jorgensen and Olensen, 2001). First, the remoteness of the Azores could have limited the number of colonizing events, restricting competition and predation from well-established organisms (Sjögren, 1973). Second, the Azores offer a rich variety of ecological habitats for colonizers, including coastal, humid, forest, and prairie environments (Dias, 2001). Finally, genetic drift processes associated with the colonization of oceanic islands tend to favor complexes of epistatic genes already present in the ancestral population conferring adaptation to particular environmental settings (Crow and Kimura, 1970). In situ speciation probably occurred very recently, in the Pleistocene (cf. Inda et al., 2008), exemplifying a rapid radiation event of

Macaronesian plants. This example might also serve as a useful model to understand the evolutionary processes that fostered a rapid speciation through ecological adaptation to coastal environments of mountain ancestors in the closely related continental polyploid complexes of *Festuca* sect. *Aulaxyper* (Catalán, 2006; Inda et al., 2008).

Colonization and Dispersal Routes of F. agustinii, F. francoi, *and* F. petraea

Dispersal model tests were a major tool in selecting optimal colonization routes of the Macaronesian red fescues. To deduce the ancestry of a Bayesian group, sequential analysis of increasing K models from STRUCTURE and the topology of the PCT was considered in each species.

In the Canarian *F. agustinii*, Bayesian methods suggest that the younger westernmost island populations were derived from those located in the older easternmost islands, likely from Tenerife island (Fig. 3c2). This hypothesis assumes that geological older islands were colonized before the relatively younger islands of La Palma and El Hierro but without a defined east-to-west stepping stone colonization route. The detected retrocolonization event suggested by STRUCTURE analysis and PCT from the younger island of El Hierro to the older island of La Gomera indicates that island dispersal is not dependent on linear geographic or chronological relationship as IBD and genetic distance matrix correlations had always lower values than dispersal model and genetic distance matrix correlations. It also indicates that a single island could have been colonized several times from relative different genetic pools, possibly of different evolutionary origins. The La Palma populations Fagus10 and to a lesser extent individuals of Fagus11 exhibited a decreasing proportion of membership to the eastern Bayesian group. This situation could be interpreted in two different ways in the context of a Bayesian analysis (Rosenberg et al., 2002): (i) they could represent gradations of allele frequencies between eastern and western populations and thus might be interpreted as genetic bridges between the two Bayesian groups; or (ii) they could also represent an admixture zone between both groups, in which case these populations might have resulted from a relatively recent colonization from Tenerife that hybridized with already established populations on La Palma. Dispersal models and the PCT indicate that the genetic relationships between the optimal Bayesian groups is best explained if La Palma was colonized only once from northern Tenerife. Models 1 and 4 also suggest two southward dispersal routes along the longitudinal axes of Tenerife and La Palma–El Hierro, respectively.

The PCT and STRUCTURE analyses suggest that the initial colonization for the Azorean endemic *F. francoi* could have occurred in the eastern subarchipelago of the Azores, because this region harbors the first diverging group from the common ancestor (Fig. 4a). According to the splitting order of divergence of the PCT Bayesian genetic units and the dispersal model 1 (Fig. 4b, c2), an initial westward dispersal occurred from São Miguel to

the central subarchipelago islands; a second westwards dispersal is inferred from Terceira to Flores. The STRUC-TURE analysis supported the existence of such groups (Fig. 4a, K = 2), indicating a close relationship between the putatively younger western archipelago and the central subarchipelago group. Model 1 further suggests two southwestern parallel dispersal events to the younger islands of the central subarchipelago. This would be compatible with the volcanic events associated to the recent ages of those islands, indicating fast secondary dispersals from the main island of Terceira to the newly emerged islands of São Jorge, Faial, and Pico.

In the Azorean *F. petraea*, genetic divergence from a common ancestor might have occurred in two scenarios. Scenario 1 (Fig. 5c1) suggests a bidirectional centripetal colonization from the central subarchipelago Faial or Graciosa islands. Starting from these central subarchipelago islands a hypothetical ancestral colonization event reached the proximal São Jorge and Pico islands. STRUCTURE genetic analysis (Fig 5a) indicates that the Graciosa Fpetr2 population represents a possible genetic bridge between the two Bayesian groups. Posterior dispersions started from Graciosa or Faial to the western-most Flores and the easternmost Santa María islands. The second scenario (scenario 2), involving Flores as the starting point of colonization (Fig. 5c2), is more compatible with the interspecific relationship found between an older paraphyletic *F. francoi* and a more recently derived *F. petraea*. *Festuca petraea* appears as the sister clade of Flores Ffran 7 population in the PCT (Fig. 2a). Thus, scenario 2 is supported by dispersal analysis and by infra and interspecific genetic relationships, whereas STRUC-TURE analysis is inconclusive to discriminate between the two scenarios. Model 1 dispersal analysis suggests that within the central subarchipelago of the Azores, a possible recolonization could have resulted in the connection of a younger island Faial Fpetr4 ancestor with an older Graciosa Fpetr2 population descendant. Although this suggests dispersal routes with an inverse direction to the geological age of the islands, it should be noted that the Azorean populations of *F. petraea* exist within a dynamic volcanic environment. Graciosa has been the source of eruptions with significant emission of lava and pyroclastic flows (Global Vulcanism Program [GVP], 2006), the latter with high destructive power. Consequently, it is possible that extinction of past populations of Graciosa could have been followed by a recolonization from younger island populations.

Long-Distance Oceanic Colonization and East-to-West Dispersal Hypotheses Related to the Endemic Macaronesian Flora

Our population-based phylogeographical study of the Macaronesian red fescues provides insights into the colonization and speciation processes followed by these recently evolved plants in the three Atlantic archipelagos. They could serve as models to understand similar distribution patterns observed in other endemic Macaronesian angiosperms and to assess various dispersal and ecolog-ical theories of speciation in oceanic islands. Our data indicate that multiple colonizations likely happened from mainland continental ancestors. Within species, three different colonization patterns were observed: a predominant east-to-west colonization pattern, concordant with the geological ages of the islands, was selected as the optimal model for the Azorean *F. francoi*; for the Canarian *F. agustinii* a bidirectional centripetal colonization pattern was inferred, whereas for *F. petraea* a counterintuitive direction of colonization (west-to-east) is suggested (Figs. 3 to 5; Tables 3 and 4). Whereas the geological eastern-to-western dispersal pattern recovered for *F. francoi* agrees with a likely arrival of continental founders to the more ancient and geographically close island of São Miguel, followed by the subsequent dispersal of the new species to the west, the origin and initial dispersal of *F. petraea* could have occurred in any of the three subarchipela-gos where *F. francoi* was already present. The Bayesian approaches and dispersal model 1 support an earlier origin in the central subarchipelago, though a western sub-archipelago origin (PCT; Fig. 2a) could be also possible.

These general models also varied considerably in secondary dispersals and back-colonizations, reflecting the different volcanic activities and interislands dispersal possibilities of the two archipelagos. Different dispersal scenarios have been proposed for the stepping-stone interisland population colonizations within some Macaronesian archipelagos. Hess et al. (2000) suggested independent bird-mediated dispersal patterns for the fleshy and lipid-rich fruits of *Olea europaea* in the Madeiran and the Canary Islands. The intricate dispersal routes observed within *F. agustinii* in the Canary Islands and of *F. petraea* in the Azores do not seem to be connected with migratory bird routes. Rather, they reflect a more ancient volcanism-associated island formation pattern that has been also observed in other Macaronesian endemics (e.g., *Deschampsia foliosa*: M. Sequeira and P. Catalán, unpublished data). The older and more stable Canary Isles have experienced a low number of secondary dispersals, probably due to the east-to-west geographically scaled ages of the islands, which favored the saltatory route of *F. agustinii* populations from Gran Canaria to Tenerife to La Gomera to La Palma to El Hierro (models 1 and 4). Nonetheless, the geographical proximity of the western island also allowed for potential secondary invasions from Tenerife to La Palma (models 1 to 5) and a potential back-colonization from La Palma and El Hierro to La Gomera (model 5 and models 2 and 4, respectively). In the young and highly dynamic Azores isles, secondary dispersal events have been more pronounced and have occurred in different ways. They have been manifested in both the secondary colonizations of younger islands from older islands (*F. francoi* populations; model 1) and in the back-colonization of older islands subjected to volcanism-mediated extinctions from younger islands (*F. petraea* populations, model 1) within the central subarchipelago. Also, the long geographical distances that separate the eastern, central, and western Azores subarchipelagos seem to have acted as barriers preventing any recent gene flow among them.

In agreement with Carine et al. (2004), our results show that the multiple congeneric colonizations of the Macaronesian red fescues' ancestors occurred because the three different archipelagos were colonized independently. We predict that this fact might have operated in most of the species-poor Macaronesian endemic group plants, irrespectively of the colonization time, but perhaps not of their dispersal abilities. Finally, our data concur with the window-of-opportunity hypothesis (Carine, 2005) at the population level, as suggested by the secondary back-colonization of the older island of Graciosa by younger *F. petraea* lineages after local extinction of the initial founders. Despite the advances made, there is still the paradox of why, if the Macaronesian islands are so easy to colonize, there is clearly isolation, as evidenced by the high genetic divergence observed among the Macaronesian red fescues and by the lack of genetic exchange between continent and islands and back again (e.g., *Androcymbium*: Caujapé-Castells, 2004).

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