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Speciation and Biogeography of *Erigeron* (Asteraceae) in the Juan Fernández Archipelago, Chile, based on AFLPs and SSRs

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Abstract—Oceanic islands provide many opportunities for examining modes of speciation in endemic plants, especially using modern molecular methods. Most speciation has been by either cladogenesis, usually resulting in radiated complexes, or anagenesis, yielding single transformed species. Previous genetic studies with available molecular markers, representing a limited sampling of the genome, have suggested that during cladogenesis, species accrue small genetic differences but differ dramatically in morphology. An appropriate archipelago in which to evaluate the genetic consequences of cladogenesis is the Juan Fernández Islands, a national park of Chile. This present study focuses on AFLP and SSR genetic differences among and within populations of six endemic species of *Erigeron* (Asteraceae), restricted principally to the younger island, Alejandro Selkirk (one to two million years old). Results show that three different genetic lineages exist among these species: (1) *E. rupicola* and *E. stuessyi*; (2) *E. fernandezianus*; and (3) the *E. ingae* complex (including *E. ingae, E. luteoviridis,* and *E. turricola*). The three genetic lines are distinct from each other, and each harbors considerable genetic variation. The three species of the *E. ingae* complex appear to be segregating genetically but are not yet ecologically divergent. The amount of genetic differentiation among species of *Erigeron* is less than that already documented among species of *Robinsonia* (Asteraceae) on the older island (four million years old). *Erigeron fernandezianus*, occurring on both islands, appears to have originated on Alejandro Selkirk Island and subsequently dispersed and established also on Robinson Crusoe Island.

Keywords—Astereae, biogeography, Compositae, evolution, population genetics, Robinson Crusoe Islands.

Numerous investigations have shown the utility of oceanic islands for providing opportunities for revealing patterns and processes of evolution (Whittaker and Fernández-Palacios 2007; Bramwell and Caujapé-Castells 2011). Due to the isolation of oceanic islands from source areas, plus known geological ages, the number of evolutionary and biogeographic hypotheses for the origin and diversification of endemic taxa is usually less than in more complex continental settings (Emerson 2002). Comparative studies among taxa and populations, therefore, provide data for generating detailed evolutionary hypotheses that can be tested with new molecular data.

One of the most suitable oceanic archipelagos for evolutionary and biogeographic studies is the Juan Fernández (= Robinson Crusoe) Islands, representing one of the national parks of Chile. The archipelago consists of two major islands, Robinson Crusoe (= Masatierra) and Alejandro Selkirk (= Masafuera), each approximately 50 km^2 (Stuessy 1995). The utility of these islands for interpretation of evolutionary and biogeographic events derives from their east-west orientation from the major source area (southern South America), their considerable isolation from the mainland (667 km) as well as distance between the islands (181 km), and the known geological ages (ca. four my for Robinson Crusoe and one to two my for Alejandro Selkirk; Stuessy et al. 1984). This setting suggests that there is a much higher probability of a propagule first arriving and establishing on Robinson Crusoe Island, being older and closer to the continent, than on Alejandro Selkirk Island. Once the younger island was formed, it offered additional opportunities for colonization, principally from the nearby older island, followed by establishment and another cycle of speciation. It is this geographic and geological setting, therefore, that makes this archipelago markedly suitable for biogeographic and evolutionary studies (Stuessy et al. 2005).

One genus in the archipelago, *Erigeron* (Asteraceae), consists of six endemic species: *E. fernandezianus* (Colla) Solbrig, *E. ingae* Skottsb., *E. luteoviridis* Skottsb., *E. rupicola* Phil., *E. stuessyi* Valdeb., and *E. turricola* Skottsb. This is the third most species rich genus in the islands. All species are known chromosomally as $n = 27$ (Valdebenito et al. 1992). The interesting feature is that these all occur on the younger island

(Alejandro Selkirk). It seems probable, therefore, that the genus arrived and established first on the younger island instead of the older island. This is not the most likely biogeographic hypothesis, and in fact, represents a unique situation in the archipelago. After colonization to Alejandro Selkirk Island, dispersal into different habitats led to phyletic radiation and eventually six morphologically distinct species. One of the species, however, *E. fernandezianus*, also occurs on the older island, but there it is more restricted to paths and disturbed areas, suggesting back introduction perhaps even during historical time (Valdebenito et al. 1992).

Morphological and limited nucleotide sequence data suggest that the *Erigeron* complex in the Juan Fernández Islands is monophyletic, i.e. that it originated from a single continental ancestor somewhere in South America, where the genus is known to be diverse (Solbrig 1962). Morphological phenetic analyses among 22 species of *Erigeron* in South America (Valdebenito et al. 1992) have revealed all island taxa to form a cluster, but which also includes *E. leptorhizon* from coastal Peru. Noyes (2000) carried out a molecular phylogenetic study that included many species of *Erigeron* using sequences from the internal transcribed spacer of nuclear ribosomal DNA (ITS), but sampling in the islands was restricted to only *E. fernandezianus* and *E. rupicola*. It should be pointed out that the collection of the latter taxon, *Stuessy et al. 8508*, was mistakenly listed in the ITS study (Noyes 2000) as *E. rosulatus*. This species is not known from the islands, and the voucher specimen reveals this collection number to be the endemic *E. rupicola* from Alejandro Selkirk Island. These two island endemics are monophyletic with ITS data (Noyes 2000), but a comprehensive analysis including all island species has not yet been accomplished. Because of morphological cohesiveness, however (Valdebenito et al. 1992), it is likely that the group in the islands has resulted from a single introduction.

For accurate assessments of genetic variation within and among populations, both overall genomic and specific allelic patterns can profitably be used. In the present study, both amplified fragment length polymorphisms (AFLPs; Vos et al. 1995) and simple sequence repeats (SSRs) have been chosen to reveal relationships and to suggest genetic consequences of speciation and biogeography. Numerous studies have shown AFLPs to be useful for showing genetic variation within and among populations (Tremetsberger et al. 2003; López-Sepúlveda et al. 2013a). For more explicit allelic information, SSRs, especially nuclear microsatellites, have also provided helpful insights into population variation in numerous groups (e.g. Gleiser et al. 2008; Kikuchi et al. 2009).

A number of different mechanisms of speciation, and divergence leading to speciation, have been analyzed with different techniques and in different plant and animal groups of oceanic islands (Whittaker and Fernández-Palacios 2007). One of the most studied processes is radiation (via cladogenesis), which has given rise to groups of related taxa that have evolved rapidly into different habitats and have usually undergone dramatic morphological change (Schluter 2000, 2001). Another process of speciation is anagenesis, whereby an immigrant population changes over time through mutation, recombination, and drift and eventually becomes different enough morphologically and genetically from the continental progenitor to be regarded as a distinct species (Stuessy et al. 2006). Within the endemic flora of the Juan Fernández Islands, both speciation via cladogenesis and anagenesis have occurred (Stuessy et al. 1990), and these processes have taken place on both the older and younger islands. Investigation of *Erigeron*, in comparison with groups already analyzed, provides an opportunity to examine the genetic consequences of these types of speciation.

To understand evolution in the Juan Fernández Archipelago, it would seem reasonable to investigate groups residing on the younger, rather than older, island, such as is the case with *Erigeron*. Less erosion and habitat modification have occurred on the younger Alejandro Selkirk Island, resulting in present population and genetic patterns that may more clearly reflect processes of differentiation and speciation. Furthermore, the older Robinson Crusoe Island has been the location of a permanent human settlement for several centuries and has had more human disturbances (Woodward 1969) that have negatively impacted the native vegetation.

The purposes of this study, therefore, were to: (1) reveal relationships among all endemic species of *Erigeron* in the archipelago; (2) examine biogeographic scenarios in the face of the new genetic data; and (3) evaluate patterns of genetic variation within the group with reference to modes of speciation.

Materials and Methods

Plant Material and DNA Extraction—Six endemic species of *Erigeron*, *E. fernandezianus*, *E. ingae*, *E. luteoviridis*, *E. rupicola*, *E. stuessyi*, and *E. turricola* were collected during expeditions to the Juan Fernández Archipelago in 2010 and 2011. Leaves of these species were collected in silica gel from individuals of 32 (13 in Robinson Crusoe and 19 in Alejandro Selkirk), 2, 2, 9, 2, and 3 populations, respectively as listed above, in the Juan Fernández Archipelago (Fig. 1; supplemental data). Voucher specimens of each population were deposited in the herbarium of the University of Vienna (WU), and the collection data are given in the supplemental data. Total genomic DNA was extracted by using the DNeasy 96 plant kit (Qiagen, Hilden, Germany) for both AFLP and microsatellite analyses.

AFLP Fingerprinting—We conducted AFLP analyses following the protocol of Vos et al. (1995), with modifications by Tremetsberger et al. (2003). Primer combinations suitable for genotyping were tested with 85 primer combinations for 4–16 individuals from one to four populations of each of the six species. Finally, six combinations of selective primers were selected: MseI-CAA/EcoRI-ACT (FAM); MseI-CAG/EcoRI-ATC (FAM); MseI-CTA/EcoRI-AGG (VIC); MseI-CTC/EcoRI-AAG (VIC); MseI-CAT/EcoRI-ACC (NED); and MseI-CAG/EcoRI-AGC (NED). A total of 652 individuals was analyzed from the six species (Table 1). Generated AFLP fragments were run on an automated sequencer (ABI 3130xl, Applied Biosystems, California) and scored by using the program GeneMarker ver. 1.85 (SoftGenetics LLC, Pennsylvania). An automatic panel editor for scoring was used for each selective primer combination (Curtin et al. 2007), including alleles of 150–510 base pairs, and then modified manually. The repeatability of the AFLP fragments was tested as the ratio of number of fragment differences/total number of comparisons (Bonin et al. 2004) using replication of ten percent of the total individuals. The generated matrices from each primer combination were pooled into one matrix (Wooten and Tolley-Jordan 2009) for population genetic analyses.

Microsatellites—Twelve microsatellite markers were initially isolated from species of *Erigeron* based on repeatability and scoring convenience (Takayama et al. 2012a). Four combinations of multiplex PCR amplification were performed using the 5′-tailed primer method (Boutin-Ganache et al. 2001) and a slightly modified protocol of the Qiagen multiplex PCR kit (Qiagen, Hilden, Germany) following Takayama et al. (2012a). The combinations with four different dyes were as follows: ER-G241H, ER-G7RC3, ER-CO124 with 6-FAM; ER-G3OOM, ER-HAJZC, ER-HDLDG with VIC; ER-HE8SM, RER-HANK8, ER-GZHZ0 with NED; ER-HKNVD, ER-HCHKA, ER-GUJTP with PET. A total of 753 individuals was analyzed from the six species (Table 1). The amplified fragments were run on an automated sequencer (ABI 3130xl) and scored by the program GeneMarker ver. 1.85 (SoftGenetics LLC). We were unable to obtain scorable peaks with two markers, ER-HCHKA and ER-GUJTP, and these

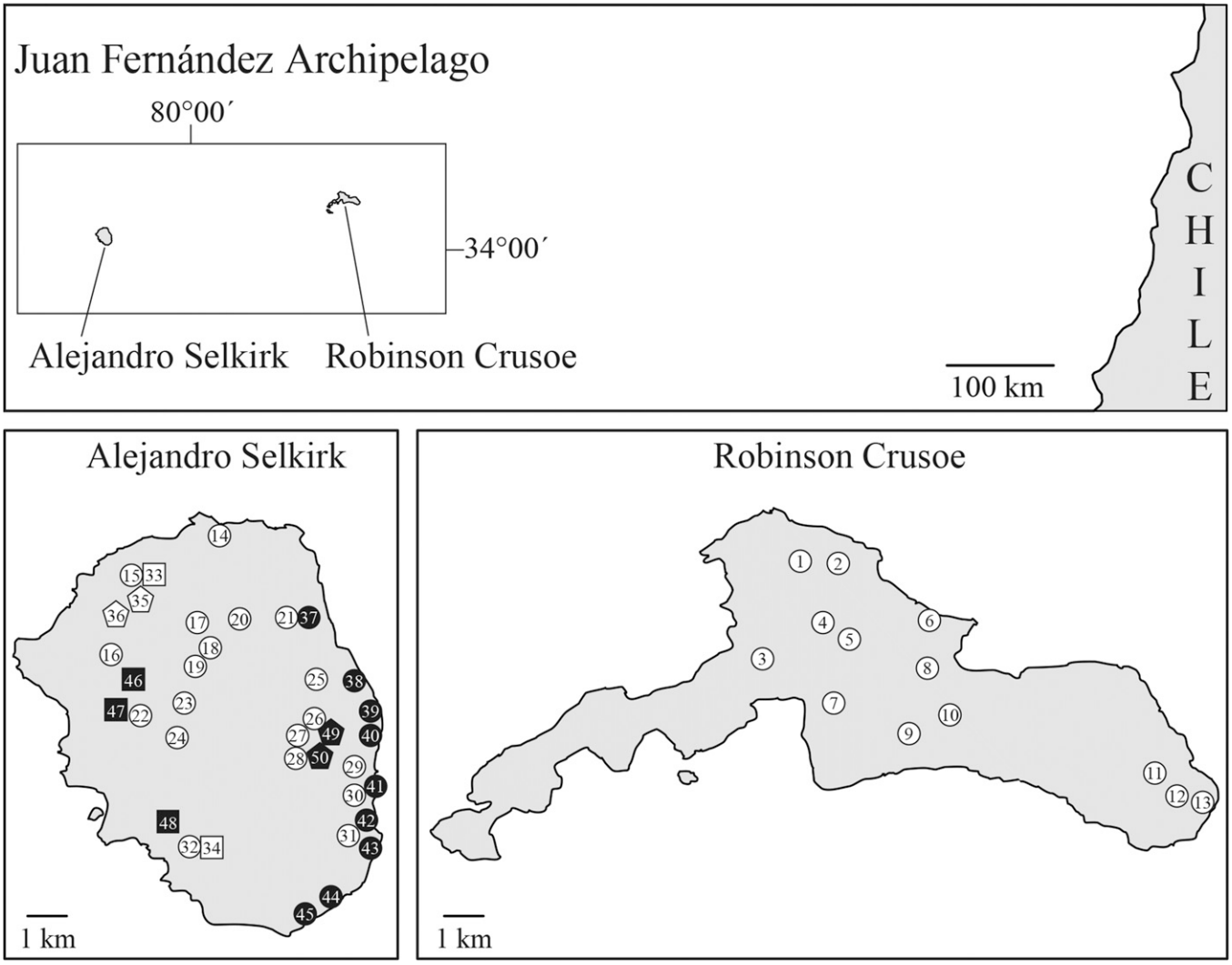

FIG. 1. Location of the Juan Fernández Archipelago and populations sampled of endemic species of *Erigeron* (Asteraceae). Open circles = *E. fernandezianus*; closed circles = *E. rupicola*; open squares = *E. ingae*; closed squares = *E. turricola*; open pentagons = *E. luteoviridis*; closed pentagons = *E. stuessyi*.

were therefore excluded from analysis. A final data matrix was generated from 10 markers and used for population genetic analyses.

***Data Analysis*—**For AFLPs, the average genetic diversity over loci in each population and species (AGDOL; the probability that two homologous band sites, randomly chosen, are different) was estimated by the program ARLEQUIN 3.5.1.2 (Excoffier et al. 2005). Other genetic diversity estimates, percentage of polymorphic bands (PPB), total number of AFLP bands (TNB), the number of private bands (NPB), and Shannon diversity index (SDI) were estimated using FAMD ver. 1.25 (Schlüter and Harris 2006) by populations and species. The rarity index (RI) (Schönswetter and Tribsch 2005) was calculated by R-script AFLPdat (Ehrich 2006). Nei-Li genetic distance among individuals was calculated from the AFLP matrix, and the inferred neighbor-net phenogram (Bryant and Moulton 2004) was based on distance using the software SplitsTree4 ver. 4.10 (Huson and Bryant 2006).

Pairwise F_{ST} between species (*E. fernandezianus* is divided into two island populations) were calculated according to Weir and Cockerham (1984). The statistical significance of departures from zero for obtaining the F_{ST} was tested using the exact test with 10,000 permutations in ARLEQUIN 3.5.1.2 (Excoffier et al. 2005). An analysis of molecular variance (AMOVA) was also implemented with ARLEQUIN 3.5.1.2 (Excoffier et al. 2005) to estimate genetic variation among and within populations in each species and also in all endemic species of *Erigeron*. Significance tests of the variance components were performed by 1,023 permutations. A Bayesian clustering method (Pritchard et al. 2000; Falush et al. 2003) to examine genetic structure and delimitation among species and populations was implemented in STRUCTURE 2.3.3 (Pritchard et al. 2000; Falush et al. 2007; Hubisz et al. 2009) using an admixture model with correlated allele frequency (after the *F*-model; Falush et al. 2003). Ten replicate runs were performed with the number of steps being 100,000, with 50,000 iteration runs in each *K* from 1–10. The highest level of structure was inferred from a posterior probability of the data for a given *K* and ΔK value (Evanno et al. 2005) using the structure harvester (Earl and von Holdt 2012).

For microsatellites, significances of linkage disequilibrium (LD) and deviation from a Hardy-Weinberg equilibrium (HWE) between loci in each population were tested with a Markov chain method with 10,000 dememorisation steps, 1,000 batches, and 500 iterations per batch, using the program GENEPOP 4.0 (Raymond and Rousset 1995). The null allele frequency in each marker was estimated using the algorithm provided by Brookfield (1996) in Micro-Checker 2.2.3 (van Oosterhout et al. 2004). The genetic diversity estimates, observed proportion of heterozygotes (H_O), expected proportion of heterozygotes (H_E), number of alleles per locus (N_A), allelic richness (A_R), and inbreeding coefficient (F_{IS}), were calculated for each population and species using FSTAT 2.9.3.2 (Goudet 1995). Allelic richness was calculated for each population and species, standardized by one and ten individuals, respectively, using the rarefaction method (Hurlbert 1971). The unbiased expected proportion of heterozygotes (μH_E), the number of private alleles (N_{PA}), and the number of locally common alleles (N_{LCA}) were estimated by GENALEX 6 (Peakall and Smouse 2006). A neighbor-joining tree based on D_A genetic distance among populations (Nei et al. 1983) was inferred by the program Populations 1.2.30 (Langella 1999). Pairwise F_{ST}, AMOVA, and STRUCTURE were estimated by the same methods as with AFLPs.

Results

Properties of AFLPs and Microsatellites—Among species of *Erigeron*, we found 444 AFLP bands, of which 100% were polymorphic. The percentages of polymorphic bands (PPB) within each species were 97.5%, 61.3%, 61.5%, 81.8%, 66.7%, and 49.3% in *E. fernandezianus*, *E. ingae*, *E. luteoviridis*, *E. rupicola*, *E. stuessyi*, and *E. turricola*, respectively (Table 2). The reproducibility of the AFLP bands was 96.2%.

Ten microsatellite loci were successfully genotyped in all individuals of the six species of *Erigeron*. There were 53 cases of deviation from HWE ($p < 0.05$) after Bonferroni correction

Table 1. Summary of genetic diversity within populations of *Erigeron* in the Juan Fernández Archipelago estimated by AFLPs and microsatellites (SSRs). *N*, total number of analyzed samples; PPB, percentage of polymorphic bands; TNB, total number of bands; SDI, Shannon diversity index; AGDOL, average genetic diversity over loci; RI, rarity index; NPB, numbers of private bands; H_O, the observed proportion of heterozygotes; H_E, the expected proportion of heterozygotes; F_{IS}, fixation index; N_A, average of number of alleles; A_R, allelic richness per one individual; N_{PA}, number of private alleles; N_{LCA}, number of locally common alleles (freq. ≥ 5%) found in 25% or fewer populations. In **bold,** average of the measures.

		AFLPs							Microsatellites								
Taxon	Population	*N*	TNB	PPB	SDI	AGDOL	RI	NPB	*N*	H_O	H_E	uH_E	F_{IS}	N_A	A_R	N_{PA}	N_{LCA}
E. fernandezianus	1	16	280	54.1	57.5	0.18	0.47	0	22	0.25	0.26	0.27	0.02	2.60	1.27	0.10	0.60
E. fernandezianus	2	18	319	47.3	46.2	0.15	0.71	0	23	0.19	0.10	0.10	−0.92	1.20	1.10	0.00	0.10
E. fernandezianus	3	21	287	49.6	49.0	0.14	0.44	0	24	0.30	0.32	0.33	0.12	2.90	1.33	0.00	0.70
E. fernandezianus	4	20	320	52.3	51.8	0.16	0.67	0	20	0.18	0.26	0.26	0.36	2.70	1.26	0.00	0.70
E. fernandezianus	5	20	318	52.3	49.9	0.15	0.65	0	19	0.16	0.19	0.19	0.28	1.70	1.19	0.00	0.20
E. fernandezianus	6	19	284	46.4	47.5	0.14	0.43	0	24	0.19	0.19	0.19	0.34	2.30	1.19	0.10	0.40
E. fernandezianus	7	16	285	55.9	58.7	0.18	0.42	0	16	0.21	0.23	0.24	0.14	2.10	1.24	0.00	0.20
E. fernandezianus	8	22	331	54.5	54.0	0.16	0.62	0	23	0.21	0.26	0.26	0.32	2.60	1.26	0.10	0.40
E. fernandezianus	9	18	307	57.7	61.0	0.19	0.47	0	24	0.22	0.26	0.27	0.25	2.40	1.27	0.00	0.40
E. fernandezianus	10	16	341	68.2	66.2	0.21	0.66	0	17	0.21	0.18	0.19	0.01	1.80	1.19	0.00	0.10
E. fernandezianus	11	11	330	56.3	60.9	0.2	0.67	0	11	0.25	0.29	0.30	0.18	2.20	1.30	0.00	0.40
E. fernandezianus	12	21	330	59.7	58.4	0.17	0.65	0	24	0.18	0.19	0.19	0.23	2.10	1.19	0.00	0.50
E. fernandezianus	13	22	316	52.7	48.7	0.14	0.61	0	24	0.25	0.22	0.23	0.13	2.10	1.23	0.00	0.50
		240	**311.4**	**54.4**	**54.6**	**0.17**	**0.57**	**0**	**271**	**0.21**	**0.23**	**0.23**	**0.11**	**2.21**	**1.23**	**0.02**	**0.40**
E. fernandezianus	14	21	331	58.6	60.7	0.19	0.71	0	24	0.17	0.39	0.40	0.57	3.30	1.40	0.10	1.20
E. fernandezianus	15	7	295	34.9	42.8	0.15	0.9	0	6	0.10	0.14	0.16	0.54	1.50	1.16	0.10	0.30
E. fernandezianus	16	5	292	36.5	46.0	0.18	0.97	0	4	0.05	0.11	0.12	0.44	1.30	1.12	0.00	0.20
E. fernandezianus	17	8	291	29.7	35.3	0.12	0.88	0	10	0.09	0.09	0.10	0.09	1.20	1.10	0.00	0.20
E. fernandezianus	18	1	207	0.0	0.0	0	0	0	1	0.10	0.05	0.10	−1.00	1.10	1.10	0.00	0.10
E. fernandezianus	19	6	281	36.3	42.4	0.16	0.83	0	7	0.11	0.16	0.17	0.52	1.50	1.17	0.00	0.40
E. fernandezianus	20	5	271	29.3	37.6	0.14	0.78	0	5	0.10	0.09	0.10	0.19	1.30	1.10	0.00	0.50
E. fernandezianus	21	3	260	37.2	52.4	0.25	0.71	0	4	0.13	0.32	0.36	0.60	2.30	1.36	0.10	0.50
E. fernandezianus	22	8	328	65.3	77.7	0.27	0.82	0	10	0.24	0.42	0.44	0.48	3.30	1.44	0.20	1.30
E. fernandezianus	23	7	323	45.7	52.7	0.18	0.95	0	8	0.14	0.36	0.39	0.61	2.50	1.39	0.00	0.80
E. fernandezianus	24	3	293	32.2	46.1	0.21	0.91	0	3	0.17	0.37	0.44	0.57	2.20	1.44	0.00	0.40
E. fernandezianus	25	6	310	40.3	50.6	0.18	0.97	0	7	0.16	0.40	0.43	0.61	2.90	1.43	0.10	1.10
E. fernandezianus	26	21	356	61.5	60.4	0.18	0.81	0	24	0.21	0.32	0.33	0.51	3.10	1.33	0.00	1.00
E. fernandezianus	27	10	322	56.5	65.6	0.21	0.7	0	20	0.22	0.37	0.38	0.42	3.40	1.38	0.00	1.00
E. fernandezianus	28	13	328	56.3	58.4	0.18	0.72	0	13	0.17	0.37	0.38	0.56	2.70	1.38	0.10	0.90
E. fernandezianus	29	23	349	56.8	58.1	0.18	0.88	0	24	0.18	0.43	0.44	0.57	3.80	1.44	0.20	1.60
E. fernandezianus	30	10	329	48.7	54.4	0.18	0.94	0	12	0.22	0.46	0.48	0.61	3.10	1.48	0.10	1.20
E. fernandezianus	31	8	330	58.3	68.2	0.23	0.69	0	11	0.20	0.31	0.33	0.41	2.80	1.33	0.00	0.80
E. fernandezianus	32	7	296	45.0	52.6	0.18	0.72	0	7	0.10	0.14	0.15	0.62	1.60	1.15	0.00	0.40
		172	**304.8**	**43.6**	**50.6**	**0.18**	**0.78**	**0**	**200**	**0.15**	**0.28**	**0.30**	**0.42**	**2.36**	**1.30**	**0.05**	**0.73**
E. ingae	33	1	186	0	0	0	0	0	1	0.10	0.05	0.10	−1.00	1.10	1.10	0.00	0.30
E. ingae	34	20	305	58.11	60.0	0.17	0.62	0	24	0.20	0.31	0.32	0.32	2.40	1.32	0.10	1.10
		21	**245.5**	**29.1**	**30.0**	**0.09**	**0.31**	**0**	**25**	**0.15**	**0.18**	**0.21**	**−0.34**	**1.75**	**1.21**	**0.05**	**0.70**
E. luteoviridis	35	8	288	40.54	44.7	0.15	1.02	0	8	0.09	0.34	0.36	0.70	2.30	1.36	0.00	0.70
E. luteoviridis	36	17	321	56.76	60.0	0.19	0.98	0	17	0.03	0.19	0.19	0.75	2.10	1.19	0.20	0.60
		25	**304.5**	**48.7**	**52.4**	**0.17**	**1.00**	**0**	**25**	**0.06**	**0.26**	**0.28**	**0.73**	**2.20**	**1.28**	**0.10**	**0.65**
E. rupicola	37	19	316	62.84	60.9	0.19	0.71	1	24	0.05	0.10	0.10	0.43	1.40	1.10	0.00	0.30
E. rupicola	38	19	314	52.03	52.6	0.16	0.71	0	20	0.16	0.14	0.14	−0.11	1.40	1.14	0.00	0.40
E. rupicola	39	20	312	51.8	57.0	0.17	0.61	0	24	0.15	0.32	0.33	0.59	2.80	1.33	0.00	1.10
E. rupicola	40	11	294	47.52	53.1	0.17	0.63	0	24	0.16	0.32	0.33	0.46	2.20	1.33	0.00	0.90
E. rupicola	41	24	311	56.53	56.8	0.17	0.63	0	23	0.19	0.34	0.35	0.57	3.10	1.35	0.10	1.40
E. rupicola	42	22	309	50.68	53.3	0.16	0.64	0	24	0.10	0.26	0.27	0.52	2.40	1.27	0.00	0.80
E. rupicola	43	19	287	46.62	47.6	0.14	0.64	0	24	0.28	0.34	0.34	0.25	2.90	1.34	0.00	1.40
E. rupicola	44	20	287	48.65	48.8	0.15	0.63	0	24	0.20	0.31	0.32	0.44	3.00	1.32	0.00	1.10
E. rupicola	45	21	295	45.05	46.5	0.14	0.78	0	24	0.25	0.30	0.31	0.22	2.90	1.31	0.10	1.20
		175	**302.8**	**51.3**	**52.9**	**0.2**	**0.7**	**0.1**	**211**	**0.17**	**0.27**	**0.28**	**0.37**	**2.46**	**1.28**	**0.02**	**0.96**
E. turricola	46	4	216	31.31	44.5	0.17	0.44	0	4	0.18	0.41	0.46	0.54	2.40	1.46	0.00	1.00
E. turricola	47	5	239	27.93	36.1	0.14	0.6	0	5	0.24	0.19	0.21	−0.15	1.60	1.21	0.20	0.30
E. turricola	48	1	115	0	0	0	0	0	1	0.50	0.25	0.50	−1.00	1.50	1.50	0.00	0.50
		11	**190.0**	**19.75**	**26.9**	**0.10**	**0.35**	**0.00**	**10**	**0.31**	**0.28**	**0.39**	**−0.20**	**1.83**	**1.39**	**0.07**	**0.60**
E. stuessyi	49	8	306	66.67	82.4	0.28	0.81	0	10	0.21	0.21	0.22	0.28	1.70	1.22	0.00	0.30
E. stuessyi	50	0							1	0.10	0.05	0.10	−1.00	1.10	1.10	0.00	0.10
		8	**306.0**	**66.7**	**82.4**	**0.28**	**0.81**	**0**	**11**	**0.16**	**0.13**	**0.16**	**−0.36**	**1.40**	**1.16**	**0.00**	**0.20**

TABLE 2. Genetic diversity within endemic species of *Erigeron* with AFLPs and microsatellites. *N*, total number of analyzed samples; PPB, percentage of polymorphic bands; TNB, total number of bands; SDI, Shannon diversity index; AGDOL, average genetic diversity over loci; RI, rarity index; NPB, numbers of private bands; H_O, the observed proportion of heterozygotes; H_E, the expected proportion of heterozygotes; F_{IS}, fixation index; N_A, average of number of alleles; A_{R11}, allelic richness per eleven individuals; N_{PA}, number of private alleles; N_{LCA}, number of locally common alleles (freq. ≥ 5%) found in 25% or fewer populations.

		AFLPs							Microsatellites							
Taxon	Island	*N*	TNB	PPB	SDI	AGDOL	RI	NPB	*N*	H_O	H_E	F_{IS}	N_A	A_{R11}	N_{PA}	N_{LCA}
E. fernandezianus	Robinson Crusoe	240	403.00	90.31	70.73	0.20	0.58	1	271	0.21	0.29	0.31	4.20	2.56	0.30	0.00
	Alejandro Selkirk	172	426.00	95.27	81.10	0.23	0.81	4	200	0.17	0.50	0.72	7.50	4.23	1.40	0.00
	Both islands	412	433.00	97.52	81.73	0.23	0.68	25	471	0.20	0.40	0.64	8.00	3.75	2.20	0.00
E. ingae		21	315.00	61.26	62.03	0.18	0.62	0	25	0.20	0.34	0.55	2.90	2.35	0.10	0.00
E. luteoviridis		25	334.00	61.49	60.22	0.18	0.99	1	25	0.05	0.31	0.72	3.10	2.62	0.40	0.00
E. rupicola		175	377.00	81.76	69.53	0.20	0.67	6	211	0.17	0.36	0.57	4.40	2.98	0.70	0.00
E. turricola		10	269.00	49.32	57.57	0.19	0.50	0	10	0.24	0.53	0.57	3.40	3.40	0.20	0.00
E. stuessyi		8	306.00	66.67	82.41	0.28	0.81	0	11	0.20	0.25	0.53	2.10	2.09	0.00	0.00

across populations and loci, and 40 cases were related to positive F_{IS} and 13 were negative. The frequency of null alleles across populations varied from 0.02–1.54, with an average frequency of 0.08 in all of the ten markers. Significant LD was found in five cases, namely between ER-HAJZC and ER-HDLDG in population 8, ER-G241H and ER-HDLDG, and ER-HDLDG and ER-HANK8 in population 14, ER-HAJZC and ER-HANK8 in population 39, and ER-G241H and ER-G7RC3 in population 40 ($p < 0.05$) after Bonferroni correction. The markers and populations were not concentrated in particular combinations, making it possible to use all of the markers for further genetic analyses.

Genetic Diversity Within and Among Species—The genetic diversity estimates of AFLPs and microsatellites within populations and species are shown in Tables 1 and 2, respectively. The values of four estimates of genetic diversity within populations with AFLPs (TNB, PPB, SDI, and AGDOL) and four estimates from microsatellites (H_E, μH_E, N_A, and A_{R1}) were correlated ($p < 0.001$). No significant differences in genetic diversity among all species (*E. fernandezianus* populations from the two islands were considered as different groups) were found in AGDOL (AFLPs), H_E, and A_{R1} (microsatellites) using Kruskal-Wallis's test and Tukey's pairwise comparison ($p < 0.05$). We also tested using Student's T test the significance of the genetic estimate between populations of *E. fernandezianus* on the two different islands, but no significant differences ($p < 0.05$) were observed in AGDOL and H_E between them.

Genetic Divergence—The results for genetic divergence of populations and species as measured by RI and NPB (AFLPs) and N_{PA} and N_{LCA} (microsatellites) are shown in Tables 1 and 2. Genetic divergences calculated by F_{ST} among species are shown in Table 3, and all values deviate significantly from zero. Averages of the F_{ST} values between species were 0.279 and 0.421 in AFLPs and microsatellites, respectively.

Genetic Structure—The results of AMOVA are shown in Table 4. In the comparison of all species of *Erigeron*, high percentages of variation were found within populations in AFLPs and among species with microsatellites. In each species (Table 4, b–g), high percentages of variation were found mostly within populations.

The neighbor-net phenogram based on AFLP data using all individuals of *Erigeron* is shown in Fig. 2, and a neighbor-joining tree based on D_A distance of microsatellites is shown in Fig. 3. In the network showing AFLP results, most individuals were clearly assorted into respective species, but there were several exceptions: in *E. rupicola* (four individuals), *E. turricola* (four), *E. ingae* (one), and *E. luteoviridis* (one). In the microsatellite tree showing relationships among populations (Fig. 3), those of *E. rupicola*, *E. fernandezianus* in Robinson Crusoe, and most of *E. fernandezianus* in Alejandro Selkirk, were recognized as clear groups, but other species showed some intergradation with each other.

The results of the Bayesian clustering method with STRUCTURE are shown in Fig. 4. The uppermost level of structure was at $K = 2$ for both AFLPs and microsatellites according to the ΔK values, but the value increased to $K = 6$ for microsatellites. This is the reason we show the genetic structure from $K = 2$ to $K = 6$. Genetic discriminations of *E. rupicola*, *E. fernandezianus* in Robinson Crusoe, and *E. fernandezianus* in Alejandro Selkirk were visible in both AFLP and microsatellites from $K = 4$. *Erigeron ingae* showed a different genetic component with $K = 5$ with microsatellites, but not with AFLPs.

DISCUSSION

Phylogenetic Relationships Among Species—Previous studies on determining relationships among species of *Erigeron* in the Juan Fernández archipelago have consisted of morphological phenetic and cladistic analyses, flavonoid

TABLE 3. Genetic differentiation (F_{ST} values) among endemic species (below: AFLPs, above: microsatellites).

	E. fernandezianus, RC	*E. fernandezianus*, AS	*E. ingae*	*E. luteoviridis*	*E. rupicola*	*E. turricola*	*E. stuessyi*
E. fernandezianus, RC		0.093	0.603	0.420	0.549	0.379	0.526
E. fernandezianus, AS	0.129		0.405	0.269	0.403	0.231	0.344
E. ingae	0.311	0.279		0.534	0.590	0.422	0.554
E. luteoviridis	0.367	0.302	0.327		0.532	0.312	0.436
E. rupicola	0.342	0.293	0.278	0.295		0.469	0.533
E. turricola	0.291	0.256	0.095	0.299	0.248		0.380
E. stuessyi	0.313	0.256	0.280	0.365	0.312	0.232	

TABLE 4. Summary of analyses of molecular variance (AMOVA), showing degrees of freedom (d. f.), sum of squares (SS), variance components, and total variance contributed by each component (%) and its associated significance (n = 1,023 permutations).

		AFLPs				Microsatellites			
Taxon	Source of variation	d. f.	SS	Variance components	Total variance (%)	d. f.	SS	Variance components	Total variance (%)
a) All *Erigeron* species	Among species	5	6,801.8	17.3	26.1	5	1,329.7	1.6	43.8
	Among populations	43	7,969.7	11.1	16.8	44	858.8	0.6	17.0
	Within populations	602	22,769.9	37.8	57.1	1,456	2,024.0	1.4	39.2
b) *E. fernandezianus*	Among islands	1	1,433.1	6.2	11.7	1	91.7	0.2	7.3
	Among populations	30	4,646.0	9.2	17.2	30	520.0	0.6	26.3
	Within populations	380	1,496.6	38.1	71.2	910	1,264.6	1.4	66.4
c) *E. ingae*	Among populations	1	57.6	10.1	20.8	1	11.2	2.5	61.5
	Within populations	19	728.9	38.4	79.2	48	75.0	1.6	38.5
d) *E. luteoviridis*	Among populations	1	66.4	2.5	6.0	1	17.8	0.8	38.2
	Within populations	23	899.3	39.1	94.0	48	59.2	1.2	61.8
e) *E. rupicola*	Among populations	8	1,629.8	8.7	19.4	8	188.2	0.472	25.3
	Within populations	166	5,974.3	36.0	80.6	413	575.2	1.393	74.7
f) *E. turricola*	Among populations	2	136.7	11.9	26.1	2	24.2	1.8	51.8
	Within populations	7	236.2	33.7	73.9	17	28.4	1.7	48.2
g) *E. stuessyi*	Among populations					1	5.8	1.3	54.6
	Within populations					20	21.6	1.1	45.4

investigations, and chromosome counts. Previous phenetic morphological investigations were done to determine relationships among endemic species of the islands and selected continental relatives (Valdebenito et al. 1992). The objectives were to ascertain if the group in the archipelago represented a monophyletic unit that evolved from a single introduction and to locate suitable progenitor taxa in the mainland. An initial phenetic analysis of 29 species of *Erigeron* from South

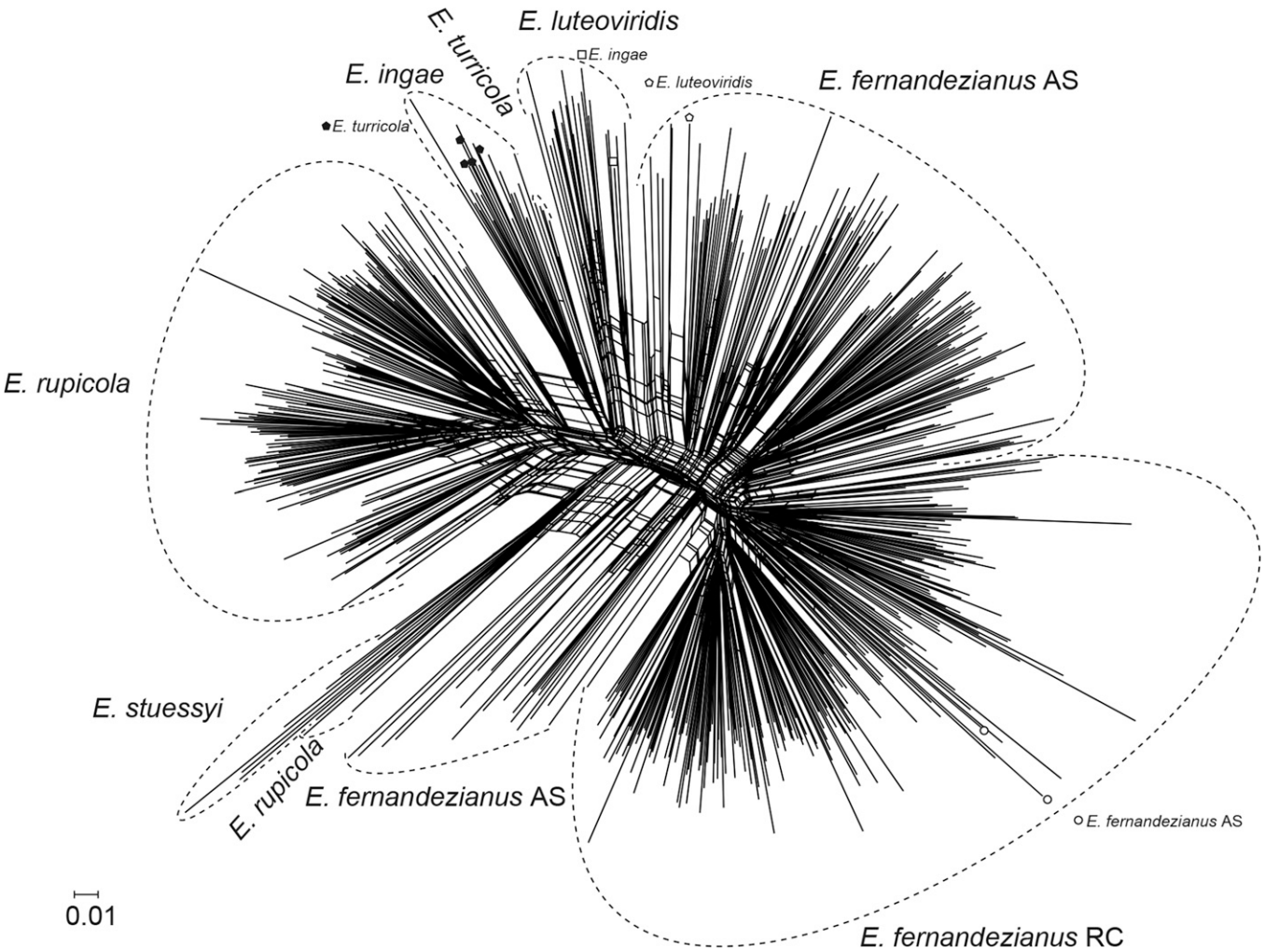

FIG. 2. Splits-Tree neighbor-net (phylogenetic network) of AFLP data showing relationships among individuals in 50 populations of endemic species of *Erigeron*. AS = Alejandro Selkirk Island; RC = Robinson Crusoe Island.

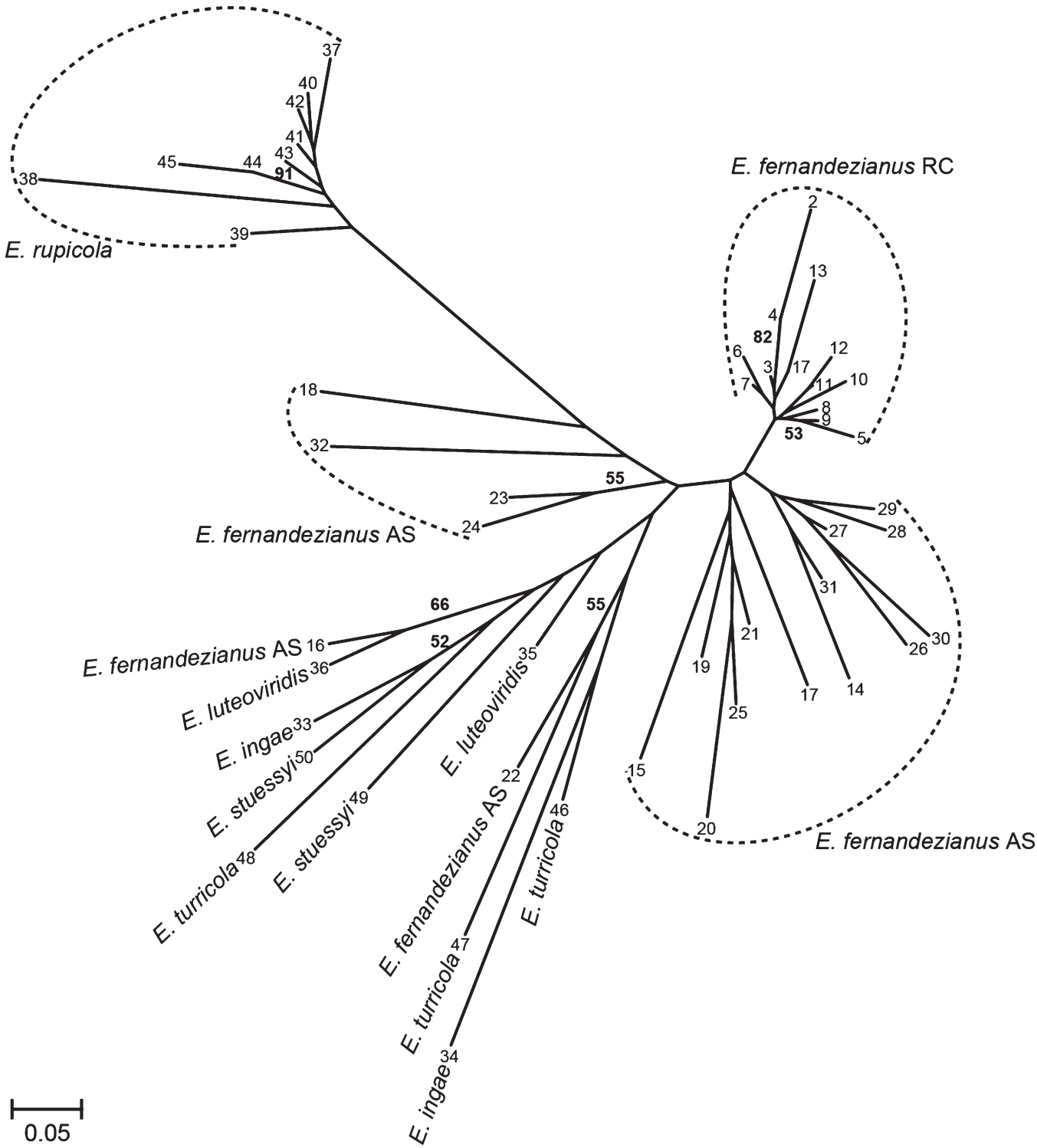

FIG. 3. Neighbor-joining tree of the 50 populations of endemic species of *Erigeron* based on distance of microsatellite data (D_A, Nei et al. 1983). Bootstrap probabilities over 50% are shown above the branches.

America (Valdebenito et al. 1992) revealed that species of sect. *Erigeron*, which are concentrated on the western side of the Andes, are more closely allied to the island endemics than those of sect. *Leptostelma* (following sectional distinctions given by Solbrig 1962; the island species are not classified definitively in the newer sectional treatment of the genus by Nesom 1989). Subsequent phenetic studies, using cluster analysis with 55 vegetative and reproductive characters (Valdebenito et al. 1992), focused on relationships among only the 22 species of sect. *Erigeron*. Significant results showed that the island taxa formed a distinct cluster, which gave support to the hypothesis that the endemic species in the islands evolved from a single introduction. Solbrig (1962) had suggested that *E. rupicola* might have had a separate introduction to the islands, but the previous (and present genetic) data do not support this view. *Erigeron*

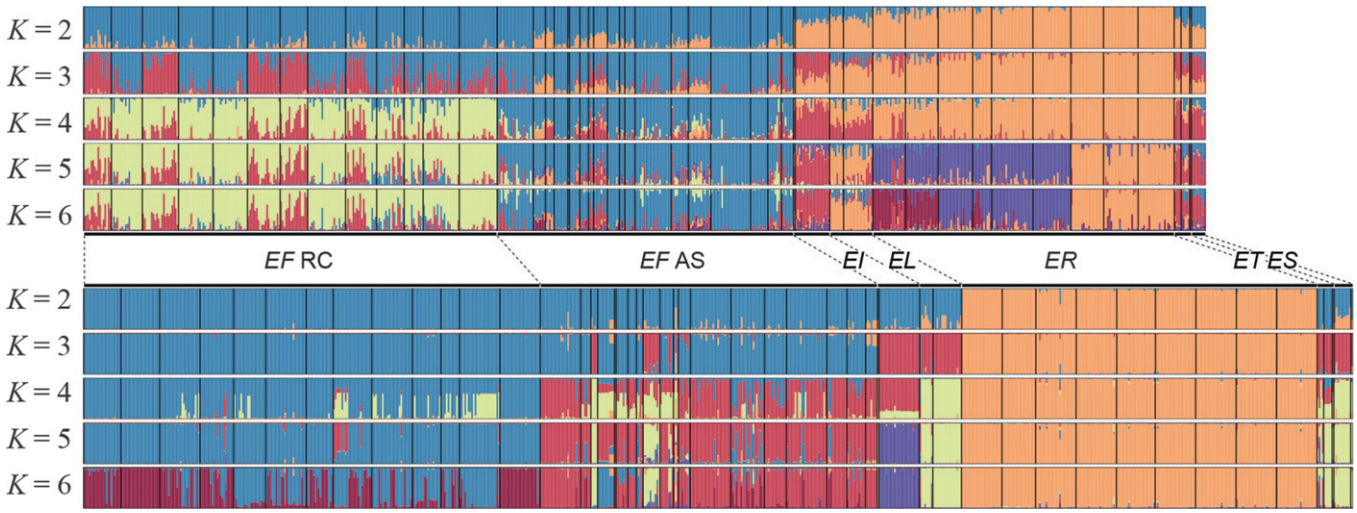

Fig. 4. Results of STRUCTURE (Pritchard et al. 2000) analyses of data from endemic species of *Erigeron*. AFLP data shown above, SSR data below. *K* = 2–6 are shown. Each individual is represented by a single vertical line broken into *K*-colored segments with lengths proportional to each of the *K*-inferred clusters. EF = *E. fernandezianus* (RC and AS, populations on Robinson Crusoe or Alejandro Selkirk Islands); EI = *E. ingae*; EL = *E. luteoviridis*; ER = *E. rupicola*; ET = *E. turricola*; ES = *E. stuessyi*.

leptorhizon from southern Peru, however, also joined this island cluster, which suggested that progenitors of the island complex might have been similar to this taxon in sect. *Erigeron*. This species inhabits dry hills (lomas) along the Pacific coast, which would be well positioned for possible dispersal to the islands, perhaps by wind or attachment to birds.

Based on the structure of relationships inferred from the phenetic analyses, morphological cladistic analyses (parsimony) were completed with focus on the island species using seven vegetative and nine reproductive characters (Valdebenito et al. 1992). *Erigeron rupicola* occurs as a small rosette herb with short flowering stalks and small solitary heads, as does also *E. stuessyi*, but this latter species has leaves that are thinner and with longer flowering stalks. *Erigeron fernandezianus* is tall, with long, dentate leaves, sometimes becoming woody at the base, and with many medium-sized heads. Morphologically the populations of *E. fernandezianus* do not differ between the two islands. The remaining species are similar, being rosette-bearing with long flowering stalks, and with many larger flowering heads. For an outgroup, a set of character states was derived from *E. leptorhizon* and five Chilean continental coastal species, using mean state values for the group. This analysis revealed two main island clades: (1) *E. rupicola* and *E. stuessyi*; and (2) *E. fernandezianus*, *E. ingae*, *E. luteoviridis*, and *E. turricola*. The species showing the lowest number of state changes from the outgroup was *E. rupicola*, which is interesting as this is the species now occurring along the coastal rocks on Alejandro Selkirk Island (50–100 m), in a fashion similar to the preferred habitat of *E. leptorhizon* in coastal Peru. The related species, *E. stuessyi*, occurs also at lower elevations, but only inside the deep, cool, and moist quebrada (= ravine) walls, whereas *E. fernandezianus* appears on steep rock walls in the canyons and ravines from below 100 m to ca. 1,200 m. In the other branch of the cladogram, *E. ingae* and *E. turricola* appear in consecutive splits, which leaves *E. luteoviridis* and *E. fernandezianus* as sister species. The species of the *E. ingae* complex all occur at higher elevations (ca. 800–1,200 m) in the fern-grassland mosaic zone, and no particular ecological differentiation has ever been documented.

Valdebenito et al. (1992) examined flavonoids from 56 populations in six endemic species of *Erigeron* in the islands (*E. ingae*, *E. fernandezianus*, *E. luteoviridis*, *E. rupicola*, *E. stuessyi*, and *E. turricola*), including 26 populations from *E. fernandezianus* on Robinson Crusoe Island and 12 populations from Alejandro Selkirk Island. In addition, five species (*E. campanensis*, *E. fasciculatus*, *E. karwinskianus*, *E. leptorhizon*, and *E. luxurians*) from continental South America were investigated. The results showed flavonols and flavones within the species analyzed. Considerable infraspecific variation prevailed, with half or fewer compounds occurring in all populations within a species. *Erigeron rupicola* and *E. stuessyi* both lack C-glycosylflavones, although the sample within the latter species was extremely limited (only one plant). Nonetheless, this does help to support their closeness based on morphological assessments. Differences in profiles between populations of *E. fernandezianus* on the two islands, however, were observed, most conspicuously with the majority of populations on Robinson Crusoe Island containing C-glycosyl-flavones and those on Alejandro Selkirk lacking them. Variation among populations of the same species, however, may be equal to or greater than among populations from different species.

Chromosome counts of $n = 27$ for all endemic species of *Erigeron* in the islands have been reported previously by Solbrig et al. (1964), Sanders et al. (1983), Spooner et al. (1987), Sun et al. (1990), and Valdebenito et al. (1992). This level, known in many species of the genus (e.g. De Jong and Nesom 1996), is apparently hexaploid; diploids at $n = 9$ and tetraploids at $n = 18$ are also common. These data again support the concept of the island endemic species having been derived from a single introduction. *Erigeron leptorhizon* has been counted as $n = 18$ (Diers 1961; Valdebenito et al. 1992), which weakens its status as a possible direct progenitor of the island taxa. Other South American species, however, have been counted as $n = 27$ (e.g. *E. ecuadoriensis*; Jansen and Stuessy 1980). This indicates that the progenitor of the immigrant to the islands was probably already at the hexaploid level. Chromosomal change after establishment of a new lineage in oceanic island archipelagos is not common (Carr 1998; Stuessy and Crawford 1998).

Relationships established by the above data and analyses can be tested by comparison with the new genetic information from AFLPs and SSRs (Figs. 2–4, Table 3). The AFLP data (Fig. 2) show four main subdivisions of the genetic diversity among individuals in populations of the six species: (1) *E. rupicola*; (2) *E. stuessyi* allied with *E. rupicola*; (3) *E. ingae*, *E. luteoviridis*, and *E. turricola*; (4) approximately one-half of the populations of *E. fernandezianus* from Alejandro Selkirk; and (5) the remaining half of the Alejandro Selkirk populations allied closely with all populations on Robinson Crusoe Island. The morphological phenetic and cladistic analyses (Valdebenito et al. 1992) showed a close relationship between *E. fernandezianus* and *E. luteoviridis*. The analyses also revealed a close tie between *E. rupicola* and *E. stuessyi* (obvious to anyone examining the morphology of these two species), and these two species are genetically closely related with AFLP data (Fig. 2). With microsatellite data (Fig. 3), however, *E. rupicola* appears different from *E. stuessyi*, which ties more closely to the *E. ingae* complex. Otherwise, the relationships deetermined from microsatellite data (Fig. 3) are similar to those from AFLPs (Fig. 2).

Understanding relationships in the *E. ingae* complex from the AFLP and microsatellite data is particularly challenging. *Erigeron ingae*, *E. luteoviridis*, and *E. turricola* cluster together into one distinct line in both, although there is considerable genetic diversity, especially evident with the microsatellite data (Fig. 3). Solbrig (1962) stressed difficulty in morphologically distinguishing *E. turricola* from *E. luteoviridis*, and he synonymized the two. The group appears, therefore, to be in the process of stabilization of consistent genetic lines. All of these taxa occur in the high elevation "alpine" zone, but there are no apparent environmental differences among the habitats of the species.

The AFLP and microsatellite data clearly point to a divergence genetically between populations of *E. fernandezianus* on the two islands (Table 3). All populations from Robinson Crusoe Island cluster together, as do those from Alejandro Selkirk Island, although there is a subdivision of populations on the younger island. There is also less genetic diversity among populations on the older island based on nearly all measures (except for H_O; Table 2). It is clear that genetic divergence has occurred between these population systems, almost as much as that seen between some of the other species, certainly more than among species of the *E. ingae* complex.

***Biogeographic Scenarios*—**The new molecular data also permit an examination of the biogeographic relationships among species of *Erigeron* in the archipelago, particularly regarding the populational separation of *E. fernandezianus* on both islands. As a general perspective, it seems extremely probable that the first immigrant of the genus successfully colonized and became established on the younger and more remote island, mainly because this is where the concentration of species diversity presently resides. This is certainly not the simplest explanation, however, because Robinson Crusoe Island was available for colonization at least two million years earlier, and it is also much closer (181 km) to the mainland. It would not have been impossible, obviously, for the progenitor of *E. fernandezianus* to have arrived first on Robinson Crusoe, speciated anagenetically, and then served as the progenitor for speciation of all the other endemic species on the younger island. Several facts argue against this alternative hypothesis, however. It would seem extremely odd that after arrival on Robinson Crusoe, the early populations of *Erigeron* would not have radiated on this island, which probably would have been larger and more ecologically diverse at its origin (Stuessy et al. 1998). This has occurred, in fact, with *Dendroseris* and *Robinsonia* with eight and seven endemic species, respectively, on Robinson Crusoe Island and both also in Asteraceae (Crawford et al. 1998). These genera also have three and one species, respectively, on Alejandro Selkirk Island (the younger island), but all these have originated anagenetically, i.e. without radiation. The three species in *Dendroseris* each have been derived from separate subgenera (Crawford et al. 1992; Crawford et al. 1998). Returning to the point, it would seem odd that the initial colonizer of *Erigeron* managed to again disperse to the younger island and only there radiate. Having separate introductions to the islands also seems unlikely because of the close genetic tie between populations on Alejandro Selkirk and those on Robinson Crusoe.

Other scenarios might be that *Erigeron* simply did not arrive to Robinson Crusoe Island until after several million years, or that earlier colonizations did not survive, or even that human impact has eliminated traces of this earlier arrival. Arguing against these hypotheses, however, is that less genetic diversity exists among all populations sampled on the older island in comparison to all those on the younger island (Table 2). One would expect that refugial populations on the older island might harbor more genetic diversity, but this is clearly not the case. The populations on the older island, therefore, may be reflecting results of a true founder effect after back migration from the younger island, perhaps even during historical time. Suggestive of this possibility is that populations of *E. fernandezianus* on the older island tend to be concentrated along the trails and in plant assemblages of aliens replacing native vegetation (i.e. disturbed areas; pers. observ.), whereas on the younger island they appear on steep rock walls in the canyons and ravines (Greimler et al. 2013). Taking all facts into consideration, it seems likely, therefore, that the genus colonized and successfully established preferentially on the younger island, rather than the usual biogeographic pattern in the archipelago of arriving first on the older island.

Erigeron in the Juan Fernández Archipelago adds another exception to the well-known "progression rule" (Funk and Wagner 1995a), whereby a colonist to an oceanic archipelago normally arrives first and speciates on the oldest island, followed by dispersal to and divergence on younger islands as they arise geologically. Numerous examples of this rule have been documented, and the most instructive have come from the Hawaiian Islands (Wagner and Funk 1995). Despite the prevalence of examples that conform to this rule, however, there are exceptions, e.g. *Clermontia* (Campanulaceae), and *Kokia* (Malvaceae). Lammers (1995) showed clearly that *Clermontia* evolved first on the younger islands, perhaps even on Hawai'i, and subsequently dispersed to and diverged on the older ones. *Kokia kauaiensis* is presently restricted to Kaua'i, but it apparently originated from a back-dispersal from the younger island of Hawai'i (Funk and Wagner 1995b). Back-dispersal is also known to have occurred infrequently in other biogeographic contexts, such as from the Canary Islands. Species are known in genera (e.g. *Aeonium*, Crassulaceae) that have been derived from northwestern Africa, radiated in the Canary Islands, and then subsequently back-dispersed to the African continent with additional divergence (Santos 1999).

Genetics of Speciation—In general, studies on the genetics of oceanic island taxa have revealed that species originating by phyletic radiation have marked morphological differences but only small genetic differences (Crawford and Stuessy 1997; Frankham 1997). Examples of well-studied groups that have shown this pattern are the Hawaiian Island silverswords (Asteraceae; Baldwin 2003), the Canary Islands *Echium* (Boraginaceae; Böhle et al. 1996), and *Robinsonia* (Asteraceae; Takayama et al. 2015a) of the Juan Fernández Islands. Studies on anagenetically derived species, on the other hand, have revealed a higher level of genetic diversity among populations. Investigations on this process have examined species of *Dystaenia* (Apiaceae; Pfosser et al. 2005) and *Acer* (Aceraceae; Takayama et al. 2012b, c) in Ullung Island, Korea, and species in *Myrceugenia* (Myrtaceae; López-Sepúlveda et al. 2013b) and *Drimys* (Winteraceae; López-Sepúlveda et al. 2015) of the Juan Fernández Archipelago. These considerations have led to a model of genetic change within species originating via these two avenues and over the ontogeny of an oceanic archipelago (Stuessy 2007). In brief, both lineages harbor approximately the same amounts of genetic diversity over time (Takayama et al. 2015b), but cladogenetically originated species, due to their partitioning of the original genetic material into different populations and eventually taxa, each contains lower levels of diversity than the single anagenetically originated species that accumulates variation through mutation, recombination, and selection and/or drift over time.

The present study examines the genetic consequences of cladogenetic speciation during radiation of *Erigeron* on the younger island (Alejandro Selkirk). The advantage of this particular system is that the process must have occurred rapidly, as the island is only one to two million years old. Furthermore, as the island is relatively young, it should have suffered less habitat modification from its original condition than has occurred on the older and more disturbed island (Stuessy et al. 1998). For understanding radiation within the archipelago, therefore, the endemic species of *Erigeron* offer the best possible system for revealing the genetic consequences of such a process. Molecular population studies have just been completed on phyletic radiation within *Robinsonia* (Asteraceae; Takayama et al. 2015a), but this genus is mostly confined to the older island. Studies on *Erigeron*, therefore, provide a chance to compare the genetic consequences of radiation on the two islands of differing ages.

Robinsonia consists of eight species, with seven confined to Robinson Crusoe Island (Takayama et al. 2015a) and one, *R. masafuerae*, having originated anagenetically on the younger island from *R. evenia*. Six species have been examined with AFLPs and SSRs in a manner similar to the results presented here for *Erigeron*. The basic difference between the genera is that there is more genetic divergence among species in *Robinsonia* than among those in *Erigeron*. The average genetic differentiation among species of the former is 0.403 with AFLPs and 0.521 with SSRs, whereas among species of the latter (with the populations in *E. fernandezianus* being considered as two groups), it is 0.279 with AFLPs and 0.428 with SSRs. This parallels the morphology, whereby more character divergence exists among species of *Robinsonia* than among species of *Erigeron* (e.g. the striking difference between *R. gracilis* and *R. gayana*). The reason for these narrower vs. broader character profiles may relate to the age of the islands and changes that have occurred within them. In particular, the *E. ingae* complex suggests that segregation for morphological and genetic features is occurring, but no ecological partitioning has yet developed. In *Robinsonia* on the older island, the genetic differences are much clearer and the genetic diversity within each species is lower. This may be due to the dual factors of older island (more time for divergence to take place) and natural loss of habitat due to subsidence and erosion, plus the overlaying human interventions during the past four centuries (Wester 1991).

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