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Morphometric, AFLP and plastid microsatellite variation in populations of *Scalesia divisa* and *S. incisa* (Asteraceae) from the Galápagos Islands

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Scalesia divisa and *S. incisa* (Asteraceae), both endemic to the Galápagos Islands, are found only on San Cristóbal, where *S. divisa* grows in the north-west of the island while *S. incisa* occurs in the north-east. At localities in between, populations with deviating individuals occur. Here we analyse the population structure of *S. divisa*, *S. incisa* and two deviating populations based on morphology, AFLP markers and two plastid microsatellite loci. The deviating populations were collected from either side of the island. In a principal components analysis based on morphological characters they appeared to be intermediate between the presumed pure species. When using a discriminant analysis, the two populations that were geographically furthest apart were best discriminated. A Mantel test showed that there was a significant correlation between morphological differentiation and geographical distance, which was also indicated in a distance tree. A second distance tree based on AFLP characters revealed the same topology, but the branches were longer. This was explained by high within-population variation, as demonstrated by AMOVA. Although only a small proportion of the total variance was explained by the between-population component, the populations were distinct enough to be separated by a discriminant analysis. A high level of misclassification was only found between one of the *S. incisa* populations and one of the deviating populations. The plastid markers supported the results obtained from AFLP. We hypothesize that the pattern of variation is the result of hybridization between two formerly isolated species.

ADDITIONAL KEYWORDS: AMOVA – cline – digital image analysis – discriminant analysis – hybridization – morphology.

INTRODUCTION

Oceanic islands are exceptional natural laboratories for studying evolutionary phenomena. Their uniqueness lies in the fact that they are often relatively small and isolated from landmasses, and that their flora and fauna must have overcome long-distance dispersal from either the mainland or other islands. As dispersal ability varies among mainland organisms, islands often comprise biotas that deviate from continental areas (Grant, 1998).

The remoteness of many islands makes them difficult to reach, and as a result their biotas are often less taxon-rich than their mainland counterparts (MacArthur & Wilson, 1967; Denslow, 2001). Consequently, unsaturated habitats are available. These may facilitate the evolution of phenotypic and ecological diversity within rapidly multiplying lineages (Schluter, 2000). The radiation may be driven by adaptations to divergent environments (Schluter, 2000) or by stochastic processes (Barrett, 1996).

When remote islands are colonized, the new populations are founded by a few individuals resulting in an immediate burst of genetic drift (Mayr, 1954).

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Thereafter, colonization is inevitably associated with long periods during which population sizes are small, which may cause considerable genetic drift followed by differentiation of the isolated populations (Brakefield, 1990; Adsersen, 1991; Barrett, 1998). These processes may result in taxa that are more or less adapted to different habitats, which may also be spatially close. If population sizes expand and no reproductive barriers have become established, hybridization between previously isolated populations may occur.

Hybridization is known from numerous radiated genera on islands (Gillet, 1972; Carr, 1985, 1995; Smith, Burke & Wagner, 1995; Francisco-Ortega, Jansen & Santos-Guerra, 1996). One striking example is the Hawaiian 'silversword alliance', with 35 natural interspecific hybrid combinations reported (Carr, 1985). In the Galápagos Islands hybridization has been discovered among Darwin's finches (Grant & Grant, 1994, 1998).

Intermediate specimens have been reported for the endemic genus *Scalesia* Arn. (Asteraceae) from the Galápagos (Eliasson, 1974). Consisting of 15 species (Eliasson, 1974; Hamann & Wium-Andersen, 1986), it is the largest endemic plant genus in the archipelago. It resembles Darwin's finches in having representatives on almost all the larger islands. Most of the taxa have restricted distribution areas, indicating that among-island dispersal is limited (Adsersen, 1990). They show significant differentiation in leaf morphological characters, exceeding the variation characteristic of some of the largest genera among the relatives of *Scalesia* that occur on the mainland (Howell, 1941). In the monographs by Cronquist (1971, in Wiggins & Porter, 1971) and Eliasson (1974) difficulties were encountered in delimiting several species. Eliasson (1974) presented several examples of intermediate specimens and questioned the existence of isolating mechanisms in the genus. One example that has turned out to be problematic is the differentiation of the species *S. divisa* Anderss. and *S. incisa* Hook.f. Although they are generally accepted as being distinct, populations with deviating individuals were found on an expedition to the Galápagos in 1999. Both species are endemic to the eastern island San Cristóbal, where they occupy an area extending from the coast inland up to approximately 150 m above sea level. *Scalesia divisa* occurs in the north-west, in the area of Caleta Sappho and Cerro Brujo, while *S. incisa* is distributed in the north-east, in the vicinity of Punta Pitt. The deviating populations occur where the species meet and contain individuals that appear to be morphologically intermediate between the species.

Scalesia divisa and *S. incisa* are both evergreen shrubs with normally rayless capitula that are either solitary or found in small groups. In *S. incisa* neuter ray florets have occasionally been found (Eliasson, 1974). Eliasson emphazised differences in leaf characters: *S. incisa* is characterized by having broad, oblong, few-toothed lobes, which are deep, up to 1/2 or 3/4 of the distance to the mid-nerve. In *S. divisa* the leaves are dentate to entire, sometimes incised to *c.* 1/3 of the distance to the mid-nerve. Eliasson (1974) also recognized differences in phyllary shape and floret number with capitula of *S. divisa* sometimes containing >100 florets and *S. incisa* < 50.

In this paper, we analyse the population structure of *S. divisa*, *S. incisa*, and populations that contain individuals intermediate between the two species. We compare the differentiation in morphological characters with genetic differentiation in AFLP and plastid microsatellite markers and discuss whether hybridization may account for the observed variation.

MATERIAL AND METHODS

DISTRIBUTION AND SAMPLING

The present distribution of the two species on San Cristóbal is as follows: more or less continuous occurrence from Caleta Sappho to Cerro Brujo (see Fig. 1), a small pocket around Population 3 (Caleta Tortuga Negra), and then a long gap between Pops 3 and 5 where no *Scalesia* has been reported. From Punta Pitt and to Bahia Rosa Blanca there is a more or less continuous belt. Pop. 1 is the most south-westerly record known. From the central area of the north-eastern part of the island there have been some findings, which were recognized as *S. divisa* (Eliasson, 1974; Hamann & Wium-Andersen, 1986).

Sampling for the present study took place in 1999. Leaf and flower material was collected from six populations as shown in Figure 1. Pops 1 and 2 were recognized as *S. divisa*, Pops 5 and 6 as *S. incisa*, while Pops 3 and 4 were deviating. Young leaves were picked

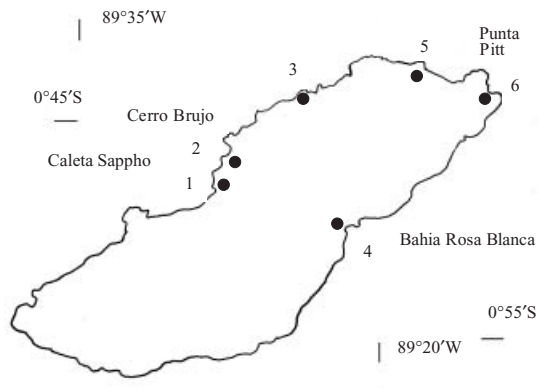

Figure 1. Sampling locations Pops 1–6 on San Cristóbal, Galápagos Islands. *S. divisa*: Pops 1, 2, S. *incisa*: Pops 5, 6, deviating populations: Pops 3, 4.

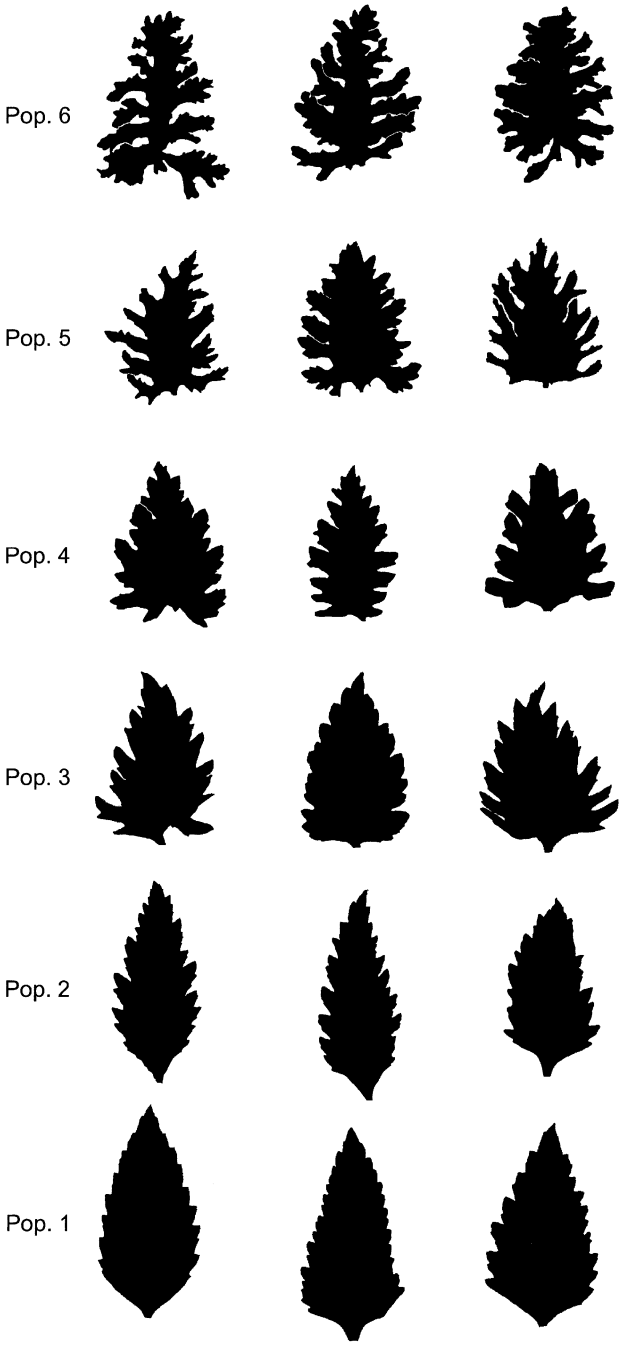

Figure 2. One leaf each from three individuals. *S. divisa* (Pops 1, 2), *S. incisa* (Pops 5, 6) deviating populations (Pops 3, 4).

and stored in silica gel for DNA analysis. Leaves judged to be of the same age were picked and pressed for morphological measurements. When possible, capitula were collected for later counts of florets per capitulum. Table 1 gives the sample size for each population.

Table 1. Sample size from each location for the different data sets. LM, leaf morphology; FC, floret counts; PM, plastid microsatellites

Pop.	LM	FC	AFLP	PM
1	*21/22	16	22	22
2	19	6	19	19
3	18	16	18	18
4	17	12	17	17
5	18	17	18	18
6	18	18	18	18
Total	111/112	85	112	112

* No whole leaf was available so only the two angle characters were measured.

MORPHOLOGICAL DATA

One randomly chosen, pressed leaf per individual was used for the morphological analyses. The leaves were scanned and seven measurements per leaf were made using Image Pro Plus. Examples of leaves from the six populations are presented in Figure 2. The measured characters were *area, major axis, minor axis, maximum diameter, minimum diameter, perimeter and roundness*. *Major and minor axis* are the lengths of the main and minor axes of an ellipse equivalent to the object (in this case, the leaves). *Maximum and minimum diameter* are the lengths of the longest and shortest lines, joining two points of the outline of the object and passing through the centroid. *Roundness* is a measure that indicates how round an image is, where a perfect circle has a roundness of 1, while more irregular objects have larger values. In addition, three characters were measured manually. *Angle 1* reflects the angle between the mid-nerve and the first strong lateral vein. *Angle 2* is the angle between the mid-nerve and the following lateral vein. *Florets* refers to the number per capitulum.

The morphological data were analysed with a principal components analysis (PCA) and a nonparametric discriminant analysis (DA; SAS, 1990). As the sample number of florets per capitulum was low for some populations, this character was omitted from the analyses and only presented with means ± standard deviation per population. The nine morphological characters were first subjected to a PCA. The variables were standardized to zero mean and variance one (the overall variance sums to 9). The transformed values of the nine characters of PCs 1 and 2 were plotted along the two component axes. The DA was based on Mahalanobis distances and on the nearest neighbour method. The classification was evaluated by cross validation.

In addition, the relationship between the populations was described with a tree also based on Mahal-

anobis distances among the populations and estimated using FITCH (Felsenstein, 1993).

Molecular data

Genomic DNA was extracted from 15 mg silica dried leaf material using DNAeasy Plant Mini Kit (Qiagen) following manufacturer's instructions with minor modifications. Between steps 4 and 5 the suggested option for very viscous lysates was applied. The samples were centrifuged for 5 min at maximum speed (13 000 rpm) and the preceding step was carried out with the supernatants. At steps 8 and 9 extra centrifugation was added when necessary.

Amplified fragment length polymorphism

AFLP was performed on 500 ng genomic DNA using the PE Applied Biosystems AFLP plant mapping kit for average sized genomes with the addition of two *Mse*I primers containing four selective bases. The procedure followed the manufacturer's protocol. The method is based on Vos *et al*. (1995), but uses *Eco*RI primers that are labelled with nonradioactive fluorescent dye. A total of 75 primer combinations was tested with *Eco*RI-based primers that contained either two or three selective bases. When testing *Eco*RI primers with two selective bases, the procedure was based on the mapping kit for small sized genomes. The *Mse*I primers had either three or four selective bases. Among all primer combinations, three were chosen that gave good amplifications and showed polymorphisms: (1) *Eco*RI(Fam)-ACT/*Mse*I-CAG, (2) *Eco*RI(Joe)-ACG/*Mse*I-CAGG, and (3) *Eco*RI(Tamra)-ACC/*Mse*I-CAGT. PCR amplifications were done in a thermal cycler (GeneAmp PCR System 9600). AFLP fragments were separated on an ABI Prism 377 genetic analyser using 5% denaturing acrylamide gels. GS-500 ROX labelled size standard (PE Applied Biosystems) was used to facilitate fragment sizing and GeneScan Analysis ver. 2.1 to estimate fragment size. The GeneScan files were further scored with Genotyper ver. 2.0, in which a binary scoring table was constructed and exported to Microsoft Excel. Only fragments that displayed good amplification in at least one individual were retained for further analysis. Fragments that differed by more than 0.5 relative migration units (bp) between samples were identified as different. We recorded 72 usable, polymorphic fragments.

Although the automatic scoring was supplemented by a visual check of each DNA profile, the final data matrix contained missing values (4.7%). To perform the following analyses these were replaced by simulated values. A '1' substituted a missing value if a random number (uniformly distributed on the interval 0–1) was equal to or smaller than the frequency of that fragment in the given population. Otherwise, a '0' substituted the missing value.

The following analyses were based on the binary matrix and carried out with Arlequin 2.000 (Schneider *et al*., 2000). Genetic diversity was estimated by two measures in the six populations. The first measure (P) equals the proportion of polymorphic markers, while the second is the mean number of differences between all pairs of haplotypes in a given population, divided by the total number of markers.

A matrix of pair-wise Euclidean distances was estimated as defined by Excoffier, Smouse & Quattro (1992). Based on this matrix an analysis of molecular variance (AMOVA) was applied to estimate variance components at different hierarchical levels: individuals within populations, populations within taxa, and populations among taxa. These are not directly comparable to the traditional F-statistics (Wright, 1951) and are therefore referred to as Φ-statistics (Excoffier *et al.*, 1992). The significance of Φ_{ST} (among populations relative to the total population) and of Φ_{SC} (among populations within taxa) was tested by 10 000 permutations of haplotypes among and within taxa respectively; that of Φ_{CT} (between taxa) by permuting populations among taxa.

The AFLP data were subjected to a nonparametric DA similar to that used for the morphological data (SAS, 1990). Relationships among populations were described with a tree based on Euclidean distances estimated using FITCH (Felsenstein, 1993).

Plastid microsatellites

Primer pairs used for the amplification of two plastid microsatellite loci in *Scalesia* were designed from sequences of the intergenic spacer region between *trn*T and *trn*L (Taberlet *et al*., 1991) and from the *psb*C–*trn*S region (Demesure, Sodzi & Petit, 1995) in *S. divisa*. The numbers of mononucleotide repeats in the two sequences from *S. divisa* were 16 and 12, respectively. The sequences of the microsatellite primers were (1) forward primer (*trn*T–*trn*L region) 5′–CAC GAT TAT TTT ATC ACG–3′ and reverse primer 5′–TTC CAC TGA TTT TCC CCT–3′ and (2) forward primer (*psb*C–*trn*S region) 5′–CGG TTC CCT CAT ACA TAT AG–3′ and reverse primer 5′–CGC CTA GCC AAG CCA GAA A–3′. The forward primers were labelled with the fluorescent dyes Joe and Fam, respectively. PCR reactions of a total volume of 20 μL were prepared (2 μL 10 × Taq buffer incl. 0.2 mM $MgCl_2$, 8 μL of a solution consisting of 0.5 mM of each dNTP, 2 μL of each primer (10 μM), 0.1 μL 5000 units/mL Taq DNA polymerase, 5 μL sterile MilliQ water and 1 μL template DNA). Amplification was conducted on a PTC 200 Peltier Thermal Cycler (MJ Research) under the following profiles. Locus 1: 4 min denatur-

ing at 94°C, followed by 35 cycles of 30 s denaturing at 94°C, 1 min annealing at 47°C and 1 min extension at 72°C, with a final extension step at 72°C for 10 min. The amplification products varied in size from 90 to 102 bp. Locus 2: as Locus 1, but with the annealing temperature at 55°C. Amplified products were from 92 to 97 bp.

The PCR products were visualized on a 2% agarose gel, and the products were diluted according to the intensity of the bands. The diluted samples were loaded on a 5% Long Ranger acrylamide gel with an internal size standard (GS-500 ROX labelled standard from PE Applied Biosystems) and run for approximately 2 h on the ABI Prism 377 genetic analyser. Fragment sizing was facilitated with GeneScan Analysis Software 2.1 and thereafter analysed with Genotyper 2.0.

Matrix comparisons

Matrix comparisons (Mantel tests) were performed in order to: (1) assess the association between genetic or geographical variation and variation in leaf morphology; (2) assess the association between genetic and geographical variation. Three matrices were constructed, one based on morphological distances among the six populations, another based on average genetic distances, and a third based on relative geographical distances. For test 1, the significance of the partial correlation coefficients was tested by permuting the rows and columns of two matrices, while keeping one matrix constant. For test 2, the significance of correlation was tested by permuting the rows and columns of one matrix, while keeping the other constant. In both cases 1000 permutations were done, each time re-computing the new partial correlation coefficient and comparing it to the observation (Smouse, Long & Sokal, 1986). Matrix calculations were carried out using Arlequin 2.000 (Schneider, Roessli & Excoffier, 2000).

RESULTS

Morphology

The means ± standard deviations of all morphological characters are presented in Table 2. In eight of the ten characters (1, 2, 4, 5, 7, 8, 9, 10) the deviating populations displayed intermediate measures when compared to the average of each population-pair representing the putative parental species (e.g. character 1: the two deviating populations are intermediate between the averages of Pops 1 and 2 and Pops 5 and 6). When comparing Pop. 1 in the north-west to Pop. 6 in the north-east there appeared to be a cline in some characters (8 and 10).

Figures 3 and 4 present the results of the PCA. In Figure 3 the symbols represent taxa; it is clear that *S. divisa* and *S. incisa* are discrete, with the two deviating populations situated between the putative parental species. In Figure 4 the symbols represent populations, revealing a cline in accordance with geographical order, where Pop. 4 is grouped in the centre together with Pop. 3 (cf. Fig. 1). PC 1 is clearly the stronger vector, accounting for 54.3% of the total variance. The second component is weaker, although when combined with PC 1, 84.4% of total variation is represented. Characters 1–5 have a high influence on PC 1. These are leaf area, the two axis measures, and the two diameter measures, all of which represent the overall size of the leaf. For PC 2, three characters make a greater contribution: roundness and the two angle measures. These influence the shape of the leaf.

As in the PCA, the two populations that are geographically farthest apart were also well separated by the DA (*S. divisa*; Pop. 1 and *S. incisa*; Pop. 6; Table 3).

Table 2. Mean ± standard deviation for morphological characters of each of the six populations. Character 1: mm^2, #2–6: mm; #8 and 9: degrees; #10: numbers

	S. divisa		Deviating populations		*S. incisa*	
	Pop. 1	Pop. 2	Pop. 3	Pop. 4	Pop. 5	Pop. 6
1. Area	770.7 ± 343.3	763.8 ± 395.1	614.7 ± 255.3	564.9 ± 219.8	365.8 ± 288.3	572.3 ± 328.9
2. Major axis	44.44 ± 11.39	42.96 ± 11.18	35.27 ± 7.40	33.60 ± 6.21	25.34 ± 9.49	32.74 ± 10.11
3. Minor axis	21.75 ± 5.06	22.21 ± 6.16	22.23 ± 4.84	21.85 ± 5.51	17.25 ± 6.67	22.62 ± 6.76
4. Max. diameter	48.61 ± 12.58	46.56 ± 12.54	38.03 ± 7.82	35.06 ± 5.80	27.40 ± 9.91	34.14 ± 9.90
5. Min. diameter	20.55 ± 4.66	20.71 ± 5.67	19.67 ± 4.13	17.32 ± 4.04	12.93 ± 4.55	13.84 ± 4.76
6. Perimeter	131.39 ± 37.73	129.50 ± 41.49	125.09 ± 34.17	143.17 ± 37.80	121.35 ± 55.29	192.89 ± 73.56
7. Roundness	1.85 ± 0.32	1.83 ± 0.48	2.11 ± 0.61	3.02 ± 0.77	3.67 ± 1.41	5.48 ± 1.33
8. Angle 1	48.41 ± 7.25	55.63 ± 11.16	58.78 ± 9.93	55.41 ± 13.46	72.22 ± 11.21	88.28 ± 8.80
9. Angle 2	58.32 ± 11.40	63.79 ± 10.22	62.06 ± 8.38	66.35 ± 12.09	70.67 ± 9.36	82.89 ± 10.08
10. Florets	102.63 ± 29.20	98.50 ± 22.10	76.75 ± 21.87	37.25 ± 12.43	37.00 ± 9.81	32.84 ± 6.40

Table 3. Discriminant analysis based on leaf morphology. The populations listed in the left most column represent the population from which the assigned individual originated. The values are the percentages by which individuals are allocated to the different populations. The diagonal in bold reflects the percentage of individuals assigned to the population of origin

	Pop. 1	Pop. 2	Pop. 3	Pop. 4	Pop. 5	Pop. 6
Pop. 1	**76**	19	5	0	0	0
Pop. 2	26	**21**	37	11	5	0
Pop. 3	6	33	**33**	22	6	0
Pop. 4	12	0	24	**47**	18	0
Pop. 5	0	6	6	17	**44**	28
Pop. 6	0	0	0	0	11	**89**

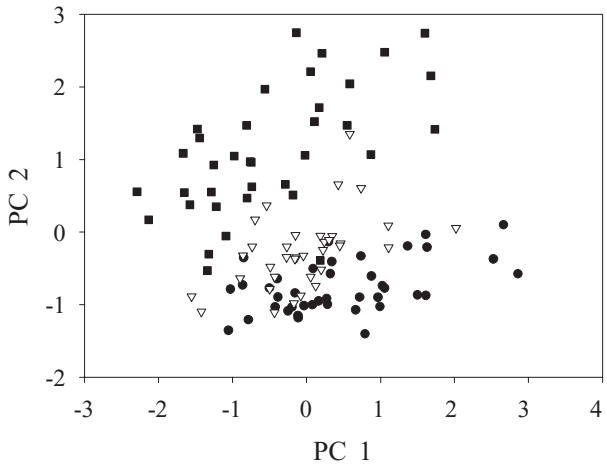

Figure 3. PCA of morphological characters (#1–9). (●) *S. divisa*; (▽) deviating populations; (■) *S. incisa*.

Individuals from these two populations were correctly classified in 76% and 89% of cases, respectively. Furthermore, the two populations were never misclassified into populations of the other species. Turning to the next population in the geographical range (Pop. 2) a high proportion of misclassification was found, with most of the misclassified individuals ending up in Pop. 3 (putative hybrid) and a smaller proportion (5%) ending up in *S. incisa* (Pop. 5). Pop. 3 was mostly classified into either *S. divisa* (Pops 1 and 2) or one of the putative hybrid populations, while Pop. 4 was more often assigned to *S. incisa* (Pop. 5) if not one of the putative hybrids. Individuals of Pop. 5 belonged mostly to their population of origin or to the other *S. incisa* population, although a relatively high proportion (17%) was assigned to the putative hybrid Pop. 4.

The distance tree (Fig. 5) reflects a geographical cline from Pop. 1 towards Pop. 6, with *S. divisa* (Pops 1, 2) and *S. incisa* (Pops 5, 6) situated farthest apart. The deviating populations are positioned between those thought to represent pure species, although relatively closer to *S. divisa* than to *S. incisa*. All populations are placed along the main branch.

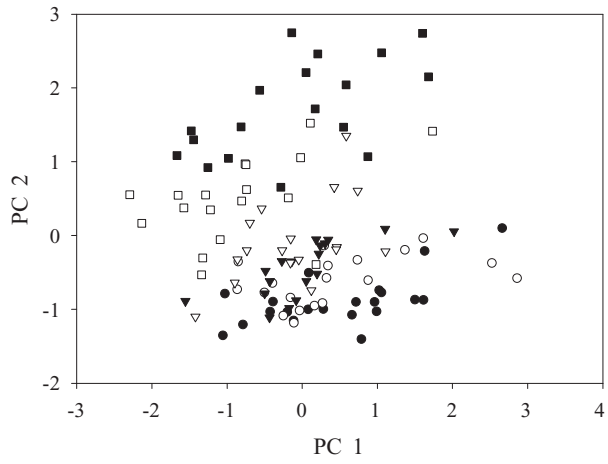

Figure 4. PCA of morphological characters (#1–9).(●) Pop. 1; (○) Pop. 2; (▼) Pop. 3; (▽) Pop. 4; (□) Pop. 5, (■) Pop. 6.

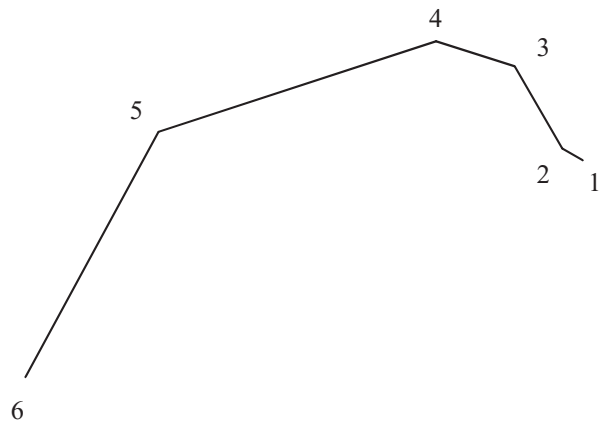

Figure 5. The relationship of populations of *S. divisa* (Pops 1, 2), *S. incisa* (Pops 5, 6), and the deviating populations (Pops 3, 4) based on morphological distances.

MOLECULAR DATA

Amplified fragment length polymorphism

None of the AFLP peaks was found exclusively in one species. The average proportion of polymorphic loci (P) was highest in one of the deviating populations and ranged from 0.764 (Pop. 2) to 0.861 (Pop. 3) (Table 4). Both putative hybrid populations displayed slightly higher diversity than the others (0.267 ± 0.138 and 0.261 ± 0.136; Table 4).

The results of the AMOVA based on AFLP variation are shown in Table 5. Most of the total variance was found within populations (~93%, $P < 0.001$). The cor-

responding Φ_{ST} value was 0.07 ($P < 0.001$). The among-population/within-taxa percentage was low (4.45%, $P < 0.001$) when compared to the within-population variance component, while the among-taxon component made only a very small contribution to the total variation (2.57%, $P = 0.064$).

In Figure 6 the relationships of the six populations are represented; the topology is identical to that of the morphological tree. With the exception of Pop. 4, each population has a long branch, in line with the high within-population variance component found in the AMOVA. As in the morphological tree, the two species are situated furthest apart, with the hybrids positioned between. The length of the branch of Pop. 3 indicates a relatively long distance to the other populations. Pop. 4 is fairly close to Pop. 5 (Table 6): these were the only populations that were not significantly different ($P = 0.091$).

The results of the DA based on AFLP characters are presented in Table 7. The proportion of correctly assigned populations was generally high (88–100%) except for Pop. 5 (33%). Misassignment occurred between Pops 4 and 5, which is in accordance with the short distance between these two populations in the tree based on Euclidean distances (Fig. 6, Table 6).

Table 4. Genetic diversity based on AFLP markers. Average proportion of polymorphic loci (*P*), and the average number of pairwise differences (± SD) per population of *S. divisa*, *S. incisa*, and the deviating taxa. Deviating taxa in bold

Population	*P*	Pairwise differences
Pop. 1	0.833	0.233 ± 0.121
Pop. 2	0.764	0.247 ± 0.128
Pop. 3	**0.861**	**0.267 ± 0.138**
Pop. 4	**0.819**	**0.261 ± 0.136**
Pop. 5	0.792	0.241 ± 0.123
Pop. 6	0.847	0.257 ± 0.134

PLASTID MICROSATELLITES

As shown in Table 8 six alleles were found in microsatellite 1. In *S. divisa*, Pop. 1 was the more variable population with three alleles, two of which were shared with Pop. 2. In *S. incisa*, Pop. 6 was more variable, also with three, whereas Pop. 5 only had one. *Scalesia divisa* and *S. incisa* had only one allele (no. 1) in common. The two deviating populations (Pops 3 and 4) carried different alleles: no. 6, found in Pop. 3, was not found in any of the others, while no. 1 in Pop. 4 was also present in Pop. 5. Microsatellite 2 was less polymorphic. Variation was only found in *S. divisa*, each of the two populations carrying two alleles. Both *S. incisa* populations carried the same allele (no.1, shared with Pop. 2 of *S. divisa*). Pop. 4 carried the same allele (no. 1) as *S. incisa*, while Pop. 3 carried no. 2, which was the most common allele in *S. divisa*. Notably, Pops 4 and 5 carried the same allele at both loci, which is in agreement with the nonsignificant distance based on AFLP markers between these two populations.

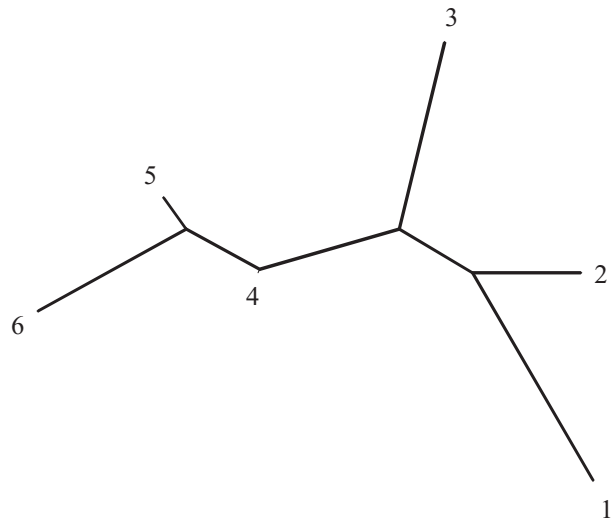

Figure 6. The relationship of populations of *S. divisa* (Pops 1, 2), *S. incisa* (Pops 5, 6), and the deviating populations (Pops 3, 4), based on genetic distances gathered from AFLP data.

Table 5. Hierarchical analysis of molecular variance based on AFLP markers among taxa (*S. divisa*/*S.incisa*/deviating taxon), among populations within taxa, and among individuals within populations. d.f., degrees of freedom; SSD, sum of squared deviations; VC, variance component; % var, percentage of variation

Source of variation	d.f.	SSD	VC	% var	*P*	Φ
Among taxa	2	52.691	0.24933	2.57	0.064	$\Phi_{CT} = 0.026$
Among populations within taxa	3	51.135	0.43122	4.45	<0.001	$\Phi_{SC} = 0.046$
Among individuals within populations	106	955.416	9.01336	92.98		
Total	111	1059.241	9.69390			

Table 6. Corrected average pairwise genetic differences among populations (percentage)

	Pop. 1	Pop. 2	Pop. 3	Pop. 4	Pop. 5	Pop. 6
Pop. 1	0	–	–	–	–	–
Pop. 2	1.38	0	–	–	–	–
Pop. 3	2.37	1.41	0	–	–	–
Pop. 4	2.00	1.29	1.35	0	–	–
Pop. 5	2.19	2.21	2.07	0.47*	0	–
Pop. 6	2.50	2.41	2.06	1.15	0.82	0

*Distance is not significant ($P = 0.091$).

Table 7. Discriminant analysis based on AFLP markers. Populations listed in the left most column represent the population from which the assigned individual originated. The values are the percentages by which individuals are allocated to the different populations. The diagonal in bold reflects the percentage of individuals assigned to the population of origin

	Pop. 1	Pop. 2	Pop. 3	Pop. 4	Pop. 5	Pop. 6
Pop. 1	**91**	9	0	0	0	0
Pop. 2	5	**95**	0	0	0	0
Pop. 3	6	0	**89**	6	0	0
Pop. 4	0	0	0	**88**	12	0
Pop. 5	0	0	6	61	**33**	0
Pop. 6	0	0	0	0	0	**100**

Table 8. Allele frequency distribution of plastid microsatellites (PM) 1 and 2 in populations of *S. divisa*, *S. incisa*, and in the deviating populations (DP)

	S. divisa		DP		*S. incisa*	
	Pop. 1	Pop. 2	Pop. 3	Pop. 4	Pop. 5	Pop. 6
PM 1						
Allele 1	0.05	0.37	0	1	1	0.33
2	0	0	0	0	0	0.22
3	0.68	0.63	0	0	0	0
4	0.27	0	0	0	0	0
5	0	0	0	0	0	0.44
6	0	0	1	0	0	0
PM 2						
Allele 1	0	0.37	0	1	1	1
2	0.59	0.63	1	0	0	0
3	0.41	0	0	0	0	0

MATRIX COMPARISONS

Test 1: no correlation was found between genetic and morphological distances ($r = -0.013$, $P = 0.540$) whereas a highly significant positive correlation was found between morphology and geography ($r = 0.843$, $P = 0.002$). Test 2: no correlation was found between genetic and geographical distances ($r = 0.282$, $P = 0.130$).

DISCUSSION

The two populations of *S. divisa* and *S. incisa* that were located furthest apart on San Cristóbal Island (Pops 1 and 6) were distinguishable by morphological as well as molecular data, which supports the general acceptance of *S. divisa* and *S. incisa* as two distinct species. However, populations of either species that were closer to one another geographically were clearly more alike and more difficult to separate. The morphologically deviating Pops 3 and 4 were generally intermediate between the two species in all analyses. For example, in the analyses based on leaf morphology, they were clearly intermediate; in the PCA they were situated between the proposed pure species, although with some overlaps.

While the DA classified the two populations that were geographically furthest apart (Pops 1 and 6) correctly, in 76% and 89% of cases, respectively, those that were geographically closer were more often misclassified. In the distance tree Pops 3 and 4 were situated between the pure species. Regarding the number of florets per capitulum, individuals from Pop. 3 were intermediate, whereas those from Pop. 4 resembled *S. incisa* (Pop. 5).

Distances based on leaf morphological characters were correlated with geographical distances. For

example, in the PCA, the populations appeared in the same order as they were collected. The distance tree based on morphological characters likewise mostly resembled that between sampled populations, strongly supported by a Mantel test ($P = 0.002$).

The distance tree based on AFLP markers showed the same topology as that based on morphology, with the deviating populations placed between the others. However, the molecular data did not show a similar correlation with geographical distances and the Mantel test was not significant. The branches in the distance tree were generally long, which may be explained by the high within-population variation found in the AMOVA, which revealed that 93% of total variation was explained by this component. The high within-population variation found in the *Scalesia* populations corresponds well to that found in outcrossing, woody perennials, based on dominant markers such as AFLP or RAPD (Nesbitt *et al*., 1995; Yeh, Chong & Yang, 1995; Russell *et al*., 1999). A previous study of the breeding system of *S. divisa* showed that the outcrossing rate, estimated in one population, was not significantly different from 1.0 (Nielsen *et al*., 2000). It has further been shown that some individuals in the close relative, *S. affinis*, completely fail to set viable embryos after self-pollination (Nielsen, Siegismund & Philipp, 2003). Outcrossing therefore seems crucial in *Scalesia* and contributes to the high within-population variation found in the *S. divisa* and *S. incisa* populations. Gene flow among populations is thus considerable and consequently the among-population differentiation is low.

Although only a small proportion of the total variation was explained by the among-population component, the populations were so distinct from each other that, with the exception of Pop. 5, they were clearly distinguished by the DA. A considerable differentiation among populations was also seen in the plastid microsatellites (Table 8). As the plastid genome is maternally inherited in Asteraceae (Corriveau & Coleman, 1988) this indicates that seed dispersal is very local. As is common in island plants (Carlquist, 1974) the achenes of *S. divisa* and *S. incisa* are without structures that enhance seed dispersability. They are glabrous, as in all species of *Scalesia*, and without awns as in most of the species (except *S. cordata* and occasionally *S. microcephala*). Their structure is thus in agreement with the suggested low fruit dispersal among populations revealed by the plastid microsatellite variation.

The between-taxon component of the AFLP suggests low differentiation between the two species. Isozyme data have revealed a general tendency for oceanic island plant genera to have a low between-species differentiation when compared to continental relatives, suggesting recent speciation (Crawford, Witkus & Stuessy, 1987). Several molecular studies based on DNA data likewise show low genetic divergence among insular species (e.g. Böhle, Hilger & Martin, 1996; Francisco-Ortega *et al*., 1996; Kim *et al*., 1996). On the Galápagos Islands the oldest rocks have been dated as *c*. 4 Myr old (Cox, 1983), so the entire evolution within *Scalesia* must have occurred within a relative short time. San Cristóbal is one of the oldest islands – Geist (1996) estimates between 2.3 and 6.3 Myr old. *Scalesia incisa* and *S. divisa* are confined to the northern areas, where the oldest dated rocks are *c.* 2.3 Myr old, although the lava presently covering the surface is much younger (Geist, McBirney & Duncan, 1986).

Geist *et al*. (1986) found evidence that San Cristóbal comprises two separate volcanoes, one in the north-east and one in the south-west. They were presumably separated by a sound. The lava in these areas has lost its primary flow features and is densely vegetated. The area in between has younger lava where the primary flow features are present and the vegetation is not as dense (Geist *et al*., 1986). Based on this information, one of the two populations of *S. divisa* (Pop. 1) was collected right on the border of the former southern volcano and the newer lava flows, whereas the two *S. incisa* populations occurred on the northern volcano. Pops 2, 3 and 4 were collected on the younger lava in between. The pattern of variation in both morphological and molecular markers may thus represent introgression between two recently diverged species. *Scalesia divisa* and *S. incisa* probably originated on each side of the mid-San Cristóbal sound, respectively, as the populations from Caleta Sappho (Pop. 1) and Punta Pitt (Pop. 6) were the most divergent in morphological as well as AFLP characters, and showed the highest plastid microsatellite diversity. Three of the populations that are situated between Caleta Sappho and Punta Pitt (Pops 3, 4 and 5) had monomorphic plastid microsatellites, which is likely to be an effect of genetic bottlenecks when new populations are established. Furthermore, the high AFLP diversity in the putative hybrid populations presumably reflects crossing events between divergent progenitors. Preliminary crossing experiments between *S. divisa* × *S. affinis* have additionally shown that interspecific crossings result in high proportions of achenes that are easily germinated. These results suggest that post–pollination barriers between the species are weak or nonexistent, thus enabling hybridization in areas where different species meet.

Even though the geographical distance between Pops 4 and 5 is relatively large, the data here presented suggest that the former may have been founded from achenes originating from the latter; they carried the same allele in both plastid microsatellites. In addition, they were the only two populations that had a

nonsignificant pair-wise distance and they were weakly discriminated based on AFLP markers. They also had the same number of florets per capitulum: 37.3 ± 12.4 and 37.0 ± 9.8, repsectively. In the tree based on leaf morphological characters Pop. 4 is, however, closer to *S. divisa*, which supports a hybrid status.

Establishing the origin of Pop. 3 is not as straightforward. Concerning the plastid microsatellites, we found that in one system it shared an allele with both putative parents, while in the other it was monomorphic for an allele not found in any other population and thus either a rare allele now lost in the other population/s or a new mutation. In all analyses, Pop. 3 appeared clearly intermediate between the two species.

Clinal variation in morphological traits may in some cases be explained by an ecological gradient, e.g. in humidity. Brochmann *et al*. (1995) studied the morphological variation in subspecies of *Frankenia ericifolia* (Frankeniaceae). Two of the subspecies showed a genetically based, clinal morphological variation parallel with a gradient in humidity ranging from arid to semiarid. The authors suggested that the clines were of primary origin, basing their hypothesis on the fact that seed set was equally good in all individuals and that cultivated offspring showed the same level of genetically based morphometric variation. We cannot completely exclude the possibility that the cline observed in *S. divisa* and *S. incisa* is primary, for instance caused by differences in lava composition. However, the pattern of variation found in the neutral genetic markers was similar to the one based on morphology. Therefore, the variation in leaf morphology is not likely to reflect adaptations to a divergent environment and we are tempted to believe that the observed pattern reflects introgression between two previously geographically isolated species.

It has generally been believed that hybridization has a minor influence on the evolution of island plants (Humphries, 1979; Ganders & Nagata, 1984). However, congeneric endemic species often lack genetic barriers to prevent hybridization (Crawford *et al*., 1987; Baldwin *et al*., 1998), and several examples of hybridization between island taxa have been reported [e.g. *Scaevola* (Goodeniaceae): Gillet (1972); the silversword alliance (Asteraceae): Carr (1985, 1995); *Cyrtandra* (Gesneriaceae): Smith *et al*. (1995); *Argyranthemum* (Asteraceae): Francisco-Ortega *et al*. (1996)]. Archipelagos are forums for recent radiations where interspecific barriers may not have been established. Therefore, hybridization may be more significant than formerly believed (Grant, 1998), particularly on geologically unstable islands such as volcanic islands (Carr, 1995).

It therefore seems likely that the pattern of variation found among populations of *S. divisa* and *S. incisa* is due to hybridization events. A common ancestor presumably colonized San Cristóbal and the two species diverged, settling around the two volcanoes in the north-east and south-west. The populations have expanded and are now found on younger lava, as a result of which the probability of interspecific pollination has increased greatly. As no genetic barriers seem to have been established, the production of hybrids is thus possible. If the pattern of variation found is indeed independent of the environment as hypothesized, the continuous gradual mixing of genes between *S. divisa* and *S. incisa* may eventually lead to the two species merging.

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