

The importance of phylogeny and ecology in microgeographical variation in the morphology of four Canarian species of *Aeonium* (Crassulaceae)

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The relative importance of natural selection in the diversification of organisms can be assessed indirectly using matrix correspondence. The present study determines the environmental and genetic correlates of microgeographical variation in the growth form, leaf form and flower morphology in populations of four *Aeonium* species from section *Leuconium* using partial regression methods. The phylogeny of the four species and the other 12 species in the section was deduced from amplified fragment length polymorphism (AFLP). Pubescence of floral organs and flower size correlate with the phylogeny while traits related to growth form, leaf form, flower construction and inflorescence size correlate with ecological factors. The variation in the latter four traits may therefore reflect selection by current ecological conditions while variation in pubescence and flower size may reflect historical events like neutral mutations, founder events and drift. Additionally, the morphological analyses revealed a large amount of variation in all traits within populations. This suggests a possible influence of microhabitat on the variation in morphology of *Aeonium* in the Canary Islands. © 2002 The Linnean Society of London, *Biological Journal of the Linnean Society*, 76, 521–533.

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INTRODUCTION

Oceanic islands and archipelagos have long held a fascination for biologists, and the study of island organisms has been important for development of the theory of evolution (e.g. Darwin, 1859; Wallace, 1869; Baker, 1955). Prominent examples of such studies are of the morphologically diverse and species-rich animal and plant genera that have been coupled to mechanisms of adaptive radiation. These groups are thought to provide some of the strongest evidence for the importance of natural selection in evolution. Focusing on plants, adaptive radiation has been suggested in the Hawaiian *Cyrtandra* (Wagner, Herbst & Sohmer, 1990), *Cyanea* (Givnish *et al.*, 1995), and silverswords

(Baldwin & Robinchaux, 1995; Baldwin, 1997) and the Canarian *Argyranthemum* (Francisco-Ortega *et al.*, 1997), *Echium* (Böhle, Hilger & Martin, 1996) and *Sonchus* (Kim *et al.*, 1996). These genera are characterized by having many species that occur in restricted, non-overlapping habitats with very different vegetative and/or reproductive traits. This may demonstrate their ability to adapt morphologically to a variety of ecological conditions. Deduction of patterns of island colonization and dispersal within and between islands from the phylogeny may reveal possible routes of speciation in these genera. These may have come about through colonization of similar habitats of different islands followed by diversification due to isolation (e.g. in Canarian *Argyranthemum* (Francisco-Ortega *et al.*, 1996)), or through radiation into different habitats of the same island (e.g. in the Hawaiian silverswords (Baldwin & Robinchaux, 1995)). The latter phenomenon in particular invokes the importance of adaptation and natural selection

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because of the shifts in ecological conditions associated with the radiation.

Studies comparing distributional patterns with phylogeny may at their best only give us an indication of the mechanisms of evolution contributing to the diversity of plants and animals on islands. Recently, a number of studies on Canary Island lizards have assessed the importance of natural selection conditioned by current ecological conditions on islands by studying intraspecific microgeographical variations in morphological characters (Thorpe & Brown, 1989; Brown, Thorpe & baez, 1991; Thorpe, 1996). Although natural selection is not the only factor that appears to be of importance in the evolution of Canarian lizards (Thorpe & Richard, 2001) these studies identify traits that correlate with ecological variation when controlling for phylogenetic effects which can be taken as evidence of natural selection (Endler, 1986). The present study is the first to report on similar correlates of the microgeographical variation in morphological characters of island plants. Four species of the largest plant radiation in the Canary Islands, the genus *Aeonium*, are the subjects of the study.

The Canarian archipelago in the Atlantic Ocean consists of seven major islands (Fig. 1), all of which are of volcanic origin and have never been joined above sea-level (Abdel-Monem *et al.*, 1971, 1972). Geological dating of the islands infers a sequential formation of the archipelago along a longitudinal gradient starting with Fuerteventura in the east (21 Myr) and ending with El Hierro in the west (0.8 Myr) (Carracedo, 1978). Altitudinal differences within the islands and the strong north-eastern tradewinds result in pronounced climatic variation. The north coasts are humid while the southern parts of the islands are arid. This climatic gradient is most prominent on the five western islands which have the highest altitudes (3718 m at its highest on Tenerife). With the islands roughly arranged on a line perpendicular to the African west coast, an increase in longitude reflects an increase in oceanic climate (e.g. lower temperature amplitude, less solar radiation, higher precipitation) (Garcia Rodriguez *et al.*, 1990).

The genus *Aeonium* (Crassulaceae) has 36 of its 42 species confined to the Canarian Archipelago. Molecular phylogenetic investigations of the genus suggest that it arose in the archipelago and later dispersed to Madeira (2 taxa), Cape Verde (1 taxon) and Africa (3 taxa) (Mes, van Brederode & Hart, 1996; Jorgensen & Frydenberg, 1999). Most of its species are single-island endemics found in restricted habitats distributed throughout an island without overlapping (Bramwell & Bramwell, 1990). The genus exhibits a number of growth forms from creeping, few-branched forms to upright unbranched forms or subshrubs and shrubs (Lems, 1960).

Closely related species of *Aeonium* may occur on geographically distant islands. Colonization events may therefore have occurred several times throughout the radiation of the genus. In this evolutionary scenario it is likely that both historical factors (e.g. founder events and genetic drift as revealed by the phylogeny) and natural selection by current ecological conditions have played important roles in the diversification of the genus. Each of these factors will result in different patterns of phenotypic variation within and between species. Where phylogeny is important, phenotypic variation is predicted to correlate with evolutionary divergence between species and populations. Where ecological adaptation is important, this will be reflected in a correlation in the adapted traits to the ecological factors of selective value when phylogenetic effects are controlled for (Thorpe, 1996). Traits that do not correlate with either phylogeny or ecology may be purely random or the result of past adaptations.

The microgeographical variation in vegetative and reproductive characters in four species of *Aeonium* is studied as a first attempt to assess the importance of natural selection in the evolution of the genus. The following hypotheses are tested using partial regression methods: (1) *Phylogeny*. A small genetic distance between populations will reflect their common history if constant rates of molecular evolution can be assumed. A correlation between morphology and genetic markers supports the hypothesis that the degree of morphological resemblance is determined by the ancestral relationship of populations. (2) *Ecology*. Strong selection under current ecological conditions may not be reflected in phylogeny but may appear in morphology alone where neutral mutations and drift have had little time to occur. The responsiveness of morphological traits to ecological conditions suggests that these traits can be subject to selection.

MATERIAL AND METHODS

SAMPLE POPULATIONS

Data were collected from five populations of *A. urbicum*, one population of *A. appendiculatum* and three populations each of *A. hierrense* and *A. pseudourbicum* (Fig. 1). The four species belong to the same phylogenetic clade in the molecular phylogeny (Jorgensen & Frydenberg, 1999) which makes up section *Leuconium* (Liu, 1989). They were chosen because their unbranched growth form can be easily analysed and because their joined distribution covers a large geographical area offering a number of habitats. *Aeonium urbicum* occurs on the north and eastern parts of Tenerife. Its distributional range covers humid lowland and laurel forest in the north and dry lowland in the east and south-east of the

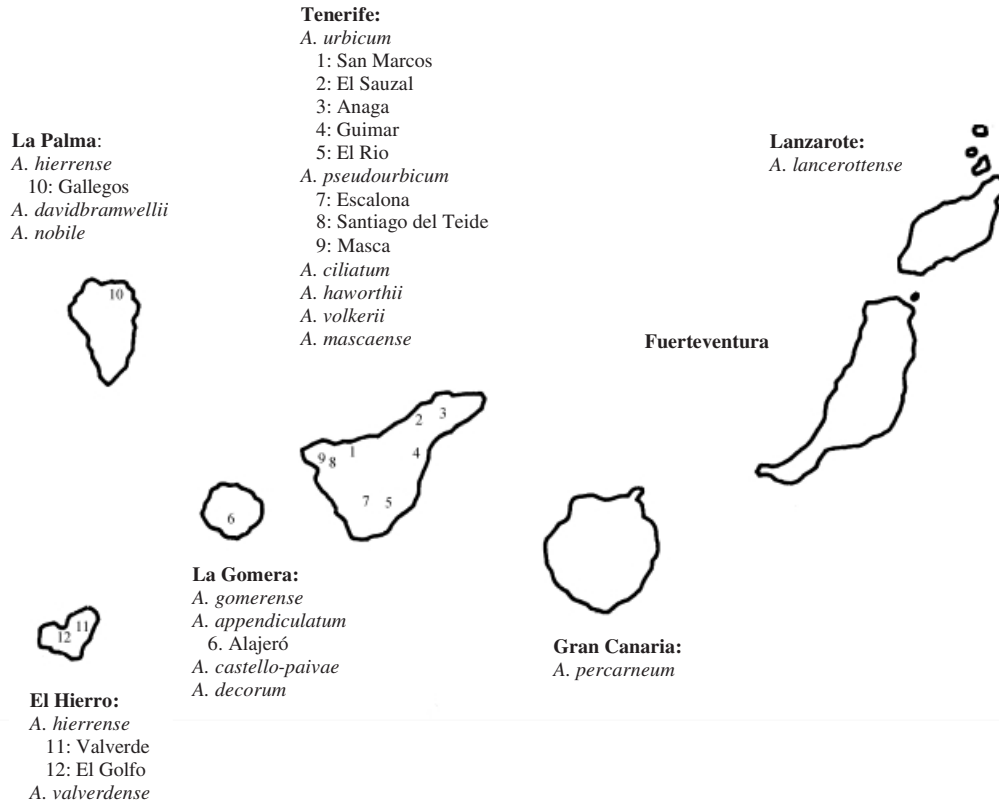


Figure 1. Islands from which the species of section *Leuconium* were sampled. Two species, *A. gomerense* and *A. mascaense*, were represented by a single accession only, obtained from the Botanical Gardens in Kew. The sample locations of populations of *A. urbicum*, *A. appendiculatum*, *A. pseudourbicum* and *A. hierrense* are numbered.

island. *Aeonium pseudourbicum* is distributed on the relatively dry parts on the west and south-west of Tenerife. *Aeonium appendiculatum* occurs on the dry southern part of La Gomera. *Aeonium hierrense* occurs in the northern humid parts of La Palma and on dry and humid parts of El Hierro.

The choice of populations reflects the geographical and ecological distribution of the four species on the four islands. Individuals of varying age were randomly selected for non-destructive analysis of growth form, leaf form and flower traits and for the collection of leaf material for DNA analyses.

MORPHOLOGY

The easily identifiable modular structure of *Aeonium* species forms the basis of the analysis of their growth form (Fig. 2). On the Canary Islands growth and extension of the stem in *Aeonium* occurs during the cold and wet months while there is no or little extension during the dry summer (June–September). Stem extension causes the formation of long internodes during the winter and very short internodes during the summer. Long and short internodes are revealed

by the distinctive leaf scars along the stem. Areas of dense leaf scars therefore identify one year's growth along the stem, i.e. growth from one dry summer to the next. Such stem parts were termed modules in the analysis of growth forms in *Aeonium*. It is, as the term implies, a structure that is continuously repeated and forms the general appearance of the plant. In the monocarpic species *A. urbicum*, *A. appendiculatum*, *A. hierrense* and *A. pseudourbicum*, modules are formed along a single shoot without any branching. After a number of years (3–13, pers. obs.), the plant forms a terminal inflorescence and dies.

In this study the growth form of the populations of *A. urbicum*, *A. appendiculatum*, *A. hierrense* and *A. pseudourbicum* is described by two models for each of the 12 populations, one describing variation in module length along the shoot, the other the age at flowering. The procedure of modelling growth form in modular species is described in detail in Jorgensen & Olesen (2000). The module length was measured in all selected individuals within a population. Variations in length along the shoot were modelled using linear regression. Regression model parameters served as descriptors of module length. In flowering individuals

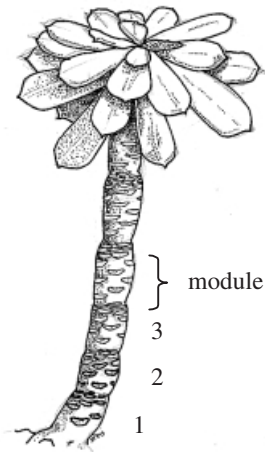


Figure 2. *Aeonium urbicum* with a module indicated. Numbering of modules starts at the base of each plant.

the number of modules along the stem were counted. The probability of flowering was modelled using logistic regression and the model parameters served as descriptors of age at death.

The succulent leaves of *A. urbicum*, *A. appendiculatum*, *A. hierrense* and *A. pseudourbicum* vary in shape from small and obovate in young individuals to long and oblanceolate in older individuals. To quantify the leaf form in a population, the largest leaf was collected from each of 19–22 individuals when the rosette was fully developed but before the onset of the dry season which causes dehiscence of the leaves. Individuals were of varying age and represented the age span of the population. Length from base to tip, maximum width and length from base to maximum width were measured. The ratio of maximum width to the length at maximum width served as a measure of leaf form. High ratio values identify obovate leaves, whereas a progression towards lower values reflects a transition to oblanceolate leaves.

The flowers of *Aeonium* conform to the general Crassulacean type by being radially symmetric, hypogynous with a corolla differentiated into petals and sepals and having two whorls of stamens and one whorl of non-fused fruits in the form of follicles. Nectariferous glands are formed at the base of each carpel. Petals in *A. urbicum*, *A. appendiculatum*, *A. hierrense* and *A. pseudourbicum* are reddish, sepals are green.

Flower traits were investigated at the time of peak flowering. The length and maximum width of 10 inflorescences per population were measured. Ten continuous variables were measured in five fully mature flowers per inflorescence to quantify the size and shape of flowers (listed in Table 1). Any presence of hairs on calyx, corolla, stamens and ovaries were

likewise recorded. The population of *A. urbicum* on Anaga was not included in this part of the morphological analyses and flower morphology was therefore studied in 11 populations only.

MICROGEOGRAPHICAL VARIATION AND SIZE CORRECTIONS

The variation in morphological characters among populations and species was assessed using nested ANOVA, Kruskal–Wallis tests and ANCOVA. Continuous reproductive characters were analysed simultaneously for differences among populations and species and a general size effect by ANCOVA with petal length as a covariate and populations nested within species. Inflorescence length served as the covariate in the analysis of inflorescence width. Variation among populations in morphological characters was a prerequisite for further analyses. Where size effects were significant, the mean of the variable when keeping size constant was subsequently used as a population mean (the intercept of separate regressions for each population). Where size effects varied significantly among populations, a population was identified by both parameters of the linear regressions on the size variable. For size variables (petal length and inflorescence length) and variables where no size effect was found, simple means were used to characterize a population.

Differences between populations in morphological characters were described by matrices of taxonomic distances calculated from the population means (or size-corrected population means) of each variable. A single distance matrix on module length was calculated from a combination of the two parameters of the regression models (each parameter standardized to a mean of zero and variance of one). The same applies to a distance matrix on flowering probabilities. The four variables describing the proportion of flowers in a population having hairs on petals, sepals, stamens or ovaries were likewise combined to a single taxonomic distance matrix. The mean of the leaf form of the three oldest leaves was used to characterize the leaf form in a population (see Results for further explanation).

PHYLOGENY

The phylogenetic relationships of species were deduced from amplified fragment length polymorphisms (AFLP). This method detects polymorphisms of genomic restriction fragments by PCR amplification (Vos *et al.*, 1995) and has proven highly useful in identifying genetic variation from the population to the species level (e.g. Travis *et al.*, 1996; Kardolus *et al.*, 1998; Muluvi *et al.*, 1999). The general advantages of the method are the relative ease with which large

amounts of genetic variation can be obtained compared to other fingerprinting methods (e.g. SSR) and its high reliability and replicability (Jones *et al.*, 1997; Rouppe van der Voort *et al.*, 1997; Waugh *et al.*, 1997).

DNA extraction

Leaves of 3–5 individuals from each of the 12 populations were collected and stored at -20°C . Additional accession of all of the other 12 species from section *Leuconium* were added to obtain a comprehensive phylogeny of the section (see Fig. 1 for species and sample locations). *Aeonium canariense* from section *Canariensia* was included as an outgroup species (Jorgensen & Frydenberg, 1999). DNA was extracted using a modified CTAB procedure (Doyle & Doyle, 1987). Concentrations were measured in a TD-700 Laboratory Fluorometer (Turner Designs) to assure standard conditions in the successive steps of the AFLP procedure.

AFLP

The reaction conditions for digestion, ligation and amplification of fragments are described in Vos *et al.* (1995). This method was adopted with some modifications: 100 μg DNA in 20 μL reaction mixture was incubated with 2.5 U EcoRI and 2.5 U MseI for 2 h at 37°C to assure a complete digest. This was followed by ligation of 2.5 pmol EcoRI-adaptors and 25 pmol MseI-adaptors. The ligation mixture was incubated at 37°C overnight.

Amplifications were performed in two steps employing primers with one and three selective nucleotides, respectively. The first step contained 30 ng of each of the two primers in 20 μL reactions with 2 μL template DNA diluted 1:10 in $1 \times \text{TE}$ buffer. The reaction mixture from the first amplification was diluted 20 times in $1 \times \text{TE}$ buffer and was used as template in the second amplification step which involved 5 ng EcoRI-primer and 15 ng MseI-primer in 10 μL reactions with 1 μL template. The PCR reactions had the same profiles as described in Vos *et al.* (1995). EcoRI primers in the second amplification step were labelled with Cy-5 fluorescent dye. Three sets of primer combinations were used to generate polymorphisms (E-CAG/M-CTT, E-CAG/M-AGG, E-CAG/M-ACG).

Three microliter amplification products were run on 7% acrylamide gels on an ALFexpress sequencer (Amersham Pharmacia Biotech) at a constant 25 W for 9 h. Co-migrating size-standards allowed alignment and sizing of the fragments. Fragments were scored as present or absent using ALFwin Fragment Analyser 1.0 (Pharmacia). Genetic distances between all species in section *Leuconium* were calculated from the fragment data using the metric of Nei & Li (1979) and used to obtain a phylogenetic tree of the section employing the UPGMA (Legendre & Legendre, 1983)

and neighbour-joining algorithms (Saitou & Nei, 1987) in PAUP* version 4.0b2. The latter method does not assume a molecular clock and any rate inconstancy across lineages can therefore be discerned from the tree. A comparison of the number of polymorphic fragments between any of the 12 populations and the outgroup *A. canariense* was likewise used to reveal differences in the relative rates of evolution (Sarich & Wilson, 1973).

The similarity measure suggested by Lynch (1990) was used to compute a genetic distance matrix of the 12 populations of *A. urbicum*, *A. appendiculatum*, *A. hierrense* and *A. pseudourbicum* only. This is a measure of interpopulation similarity calculated as the mean proportion of shared fragments between any pair of individuals in two populations and corrected by the similarity within these two populations.

CORRELATION OF MORPHOLOGY WITH PHYLOGENY AND ECOLOGY

Three factors were considered to have significant explanatory value as far as the pattern of morphological variation across populations was concerned. Species of *Aeonium* are succulent and rainfall is therefore thought to be a selective factor of prime importance. Data on mean annual rainfall on the 12 locations were obtained from Huetz de Lempis (1969) and Garcia Rodriguez *et al.* (1990). Two additional factors, latitude and longitude, reflect other undefined aspects of ecological or environmental variation. Increasing longitude reflects an increase in oceanic climate (e.g. lower temperature amplitude, less solar radiation). Latitudinal effects are related to the north–south gradient in climate within an island (e.g. temperature, solar radiation). The three factors were represented by three taxonomic distance matrices (Legendre & Legendre, 1983). Although altitude is most likely a factor of major importance for the Canarian biota this was not considered in the present analysis because the majority of the sampled populations occurred within a limited height range (500–900 m).

The variation in morphological characters in *A. urbicum*, *A. appendiculatum*, *A. pseudourbicum* and *A. hierrense* was studied simultaneously using partial regression. This method allows for hypotheses (independent variables represented as relative dissimilarity matrices) to be tested against observed patterns (morphological variables as dissimilarity matrices). Genetic and ecological distance matrices were considered against each morphological character using the backward elimination procedure on distance matrices suggested by Legendre *et al.* (1994). Analyses were carried out in the program Permute! Version 3.2 (Casgrain, 1995). When dependent variables are

simple distance matrices, this program performs significance tests of the regression parameters in the same manner as the Mantel permutation test (Mantel, 1967) while keeping independent matrix variables fixed against one another. The regression of latitudinal distances on morphological characters of Tenerife populations was carried out separately to avoid latitudinal effects arising from the different latitudinal positions of the islands. A separate Mantel test of the correlation between the genetic distance of all 12 populations and their geographical distance was likewise performed.

RESULTS

MORPHOLOGY

Growth forms as described by the module length and the flowering probability differed significantly among the 12 populations (ANCOVA: $F_{11,1207} = 7.23$, $P < 0.001$ for module length; ANCOVA: $\chi^2_{11} = 38.18$, $P < 0.001$ for flowering probability, both with module number as covariate). Parameters of the regression models for each population separately characterized a population in further analyses.

Leaf length and (log-transformed) leaf form were correlated across populations ($r = -0.83$, $P < 0.001$, $N = 240$) as were leaf age and form ($r = -0.52$, $P < 0.001$, $N = 239$), confirming the progression of leaves from short obovate in young individuals to long and oblanceolate in older individuals. Leaf form variations therefore primarily reflected the different maximum age of the populations. As a consequence, the (log-transformed) leaf form of the three oldest individuals sampled in a population was used for further analysis (Table 1). Leaf form differed significantly among populations within species (nested ANOVA: $F_{8,35} = 3.90$, $P < 0.005$) as well as among species (nested ANOVA: $F_{3,8} = 18.35$, $P < 0.005$).

Flower size (petal length, trait 5 in Table 1) differed between populations within species (nested ANOVA, $F_{8,541} = 13.48$, $P < 0.001$) and among species (nested ANOVA, $F_{3,8} = 11.44$, $P < 0.005$). Inflorescence size (inflorescence length, trait 15 in Table 1) differed between populations nested within species ($F_{7,99} = 6.79$, $P < 0.001$) but not between species ($F_{3,7} = 0.75$, $P > 0.05$). Number of petals (trait 14) likewise differed between populations (Kruskal–Wallis: $\chi^2_{10} = 148.13$, $P < 0.001$) and between species (Kruskal–Wallis: $\chi^2_3 = 103.26$, $P < 0.001$). Population means of these traits characterized a population in further analyses. Hairs were exclusively confined to the filaments in all but one population of *A. urbicum* while *A. hierrense* and *A. pseudourbicum* usually had hairs on all floral organs. No hairs were present in the flowers of *A. appendiculatum*.

All flower traits except nectary width (trait 10) had significant effects of size (ANCOVAs: $P < 0.007$ for all). The effect of size on petal width (trait 6) varied among populations (ANCOVA: $F_{10,520} = 3.81$, $P < 0.001$) whereas there were no significant differences in size effects among populations in the other traits (ANCOVA: $P > 0.05$ for all). Consequently, size-corrected means were used to characterize a population in further analyses for traits 7–9, 11–13 and 16. A combination of the two (standardized) parameters from the regression of petal width on petal length for each population was used in further analyses of trait 6 and population means were used for trait 10 where no size effect was found.

The majority of floral traits did not differ between species. In the nested analyses, sepal length, lengths of filaments, length and width of ovary and length of style (traits 7–9 and 11–13) were significantly different in mean values among populations nested within species when size was kept constant ($P < 0.0001$ for all) whereas no significant differences in mean values among species were found ($P > 0.05$ for all). Width of the inflorescence (trait 16) differed in mean value among species ($F_{3,7} = 5.90$, $P < 0.05$) but not among populations nested within species ($F_{7,99} = 1.05$, $P = 0.40$) when size was kept constant.

Some variation in morphological variables were left unexplained after regression analyses and size corrections were performed. Single regressions of module length on module number for each population had R^2_{adj} values between 0.09 and 0.53. Logistic regressions of flowering probability in each of the 12 populations had 0.70–1.00 of the flowering events correctly predicted. The regressions on petal length or inflorescence length had R^2_{adj} values between 0.01 and 0.49. Of the variables where no size-correction were performed, coefficients of variance ranged between 0.03 and 0.21.

PHYLOGENY

The amplification reactions generated a consistent pattern of fragments in the 50–500 bp size range. Above this size range fragments were more scarce and were therefore not scored. A total of 92 fragments was generated of which 47 were polymorphic in the group consisting of *A. urbicum*, *A. appendiculatum*, *A. hierrense* and *A. pseudourbicum* and an additional 35 were polymorphic when all species of the section were considered. Genetic distances between the populations of *A. urbicum*, *A. appendiculatum*, *A. hierrense* and *A. pseudourbicum* are shown in Table 2. The results of the neighbour-joining and UPGMA analyses suggest that the four monocarpic species are not closely related within section *Leuconium*. Also notable is a prominent difference between the population of *A. pseudourbicum* in Masca and the other two

Table 1. Morphological characters used in the study. Traits 7–9 and 11–13 are size-corrected values using traits 5 or 15 as covariate. Intercepts and slopes of traits 1 and 2 are parameters of regression models describing growth form. Leaf form (trait 3) are log-transformed means of the ratio of maximum width to the length at maximum width of a leaf. Where size effects were significantly different among populations (trait 6) both parameters of the linear regressions of the variable on size identified a population. For traits 5, 14 and 15 and the variable where no size effect was found (trait 10), simple means characterized a population. All continuous variables were measured in mm. Hairs (trait 3) are recorded as frequencies of occurrence in a population.

Character	<i>A. urbicum</i>				<i>A. appendiculatum</i>				<i>A. pseudourbicum</i>				<i>A. hierrense</i>			
	San Marcos	El Sauzal	Anaga	Guimar	El Rio	Alajeró	Escalona	Santiago del Teide	Masca	Gallegos	Valverde	El Golfo				
GROWTH FORM																
1. Module length:																
intercept	1.74	1.74	1.65	1.60	1.62	1.48	1.66	1.56	1.58	1.19	1.27	1.32				
slope	0.03	0.10	0.03	0.06	0.06	0.03	0.06	0.04	0.08	0.14	0.03	0.04				
2. Flowering probability:																
intercept	-0.84	-4.81	-11.04	-11.28	-13.18	-6.41	-16.83	-8.73	-12.08	-10.99	-4.67	-5.65				
slope	1.28	0.92	1.30	1.69	1.47	0.66	2.60	0.92	2.01	2.76	0.34	0.62				
3. LEAF FORM																
intercept	-0.51	-0.52	-0.49	-0.54	-0.55	-0.33	-0.55	-0.57	-0.46	-0.29	-0.04	-0.04				
FLOWER MORPHOLOGY																
4. Hairs:																
petals	0.1	0	-	0	0	0	1	0.64	1	1	0.94	1				
sepals	0.1	0	-	0	0	0	1	0.84	1	1	1	1				
filaments	1	1	-	1	0.7	0	1	1	1	1	1	1				
ovaries	0	0	-	0	0	0	1	0.64	1	1	0.9	1				
5. Petal length	8.62	8.49	-	9.46	9.35	7.22	8.16	8.15	7.83	7.70	7.85	7.97				
6. Petal width:																
intercept	1.27	1.78	-	0.90	0.98	0.16	1.43	-0.02	1.53	0.95	3.05	0.63				
slope	0.11	0.04	-	0.19	0.19	0.14	0.15	0.29	0.09	0.02	-0.11	0.15				
7. Sepal length																
intercept	2.82	2.05	-	-1.98	4.22	1.91	1.03	0.93	2.02	2.10	2.75	1.19				
8. Filament length:																
inner whorl	1.09	2.78	-	2.95	2.05	1.32	3.12	4.33	3.54	3.38	2.59	2.27				
outer whorl	4.12	3.71	-	1.91	4.18	1.94	4.15	4.52	4.04	4.63	2.95	2.48				
9. Nectary length																
intercept	0.26	0.22	-	0.08	0.69	0.30	0.24	0.51	0.45	0.56	0.64	0.58				
slope	0.77	0.92	-	0.91	1.01	0.84	0.96	0.90	0.95	0.91	0.83	0.73				
10. Nectary width																
intercept	1.38	1.60	-	2.10	2.14	2.05	1.60	1.20	1.90	2.26	0.92	2.10				
slope	1.23	1.33	-	0.37	1.00	1.51	1.33	1.11	1.27	1.11	1.04	0.37				
11. Ovary length																
intercept	2.44	1.17	-	1.81	2.27	4.53	1.17	2.51	2.56	2.89	1.08	1.81				
slope	8.04	7.90	-	8.88	8.36	7.86	8.21	8.56	8.38	7.50	7.46	7.98				
12. Number of petals																
INFLORESCENCE																
15. Length	366	428	-	320	284	287	279	337	240	375	249	359				
16. Width	298	107	-	247	159	39	63	74	125	3	-10	124				

Table 2. Genetic distances based on AFLP of 3–5 individuals per population. Distances were calculated from the metric by Lynch (1990)

Population	2	3	4	5	6	7	8	9	10	11	12
<i>A. urbicum</i>											
1. San Marcos	0.103	0.087	0.170	0.152	0.373	0.200	0.143	0.230	0.278	0.298	0.293
2. El Sauzal		0.040	0.112	0.112	0.174	0.191	0.123	0.232	0.128	0.165	0.157
3. Anaga			0.116	0.132	0.258	0.204	0.117	0.187	0.245	0.285	0.281
4. Guimar				0.070	0.207	0.184	0.132	0.184	0.209	0.254	0.235
5. El Rio					0.294	0.159	0.090	0.196	0.259	0.280	0.276
<i>A. appendiculatum</i>											
6. Alajeró						0.345	0.309	0.289	0.313	0.352	0.326
<i>A. pseudourbicum</i>											
7. Escalona							0.159	0.173	0.226	0.266	0.252
8. Santiago del Teide								0.111	0.176	0.195	0.219
9. Masca									0.142	0.145	0.191
<i>A. hierrense</i>											
10. Gallegos										0.036	0.069
11. Valverde											0.026
W12. El Golfó											

populations of the same species. The two analyses agree to a large extent on the relative positions and groupings of the 12 populations used in the morphological investigation whereas the positions of *A. gomerense*, *A. nobile* and *A. haworthii* differ between the two trees. Only the neighbour-joining tree is shown here (Fig. 3). In general, however, these phylogenies should be interpreted with caution because of low bootstrap values at the basal branches of the trees. For the same reason I use genetic distances as a proxy for phylogenetic distances in further analyses under the assumption of equal rates of molecular evolution. This assumption is supported by the degree of similarity in branch lengths of the 12 populations in the neighbour-joining tree as well as the large degree of overlap in ranges of polymorphic fragments between a population and the outgroup (Fig. 3). These ranges are determined by the minimum and maximum numbers of polymorphic fragments and are relatively large in many of the populations investigated.

CORRELATION OF MORPHOLOGY WITH PHYLOGENY AND ECOLOGY

Genetic distance between populations correlated significantly with geographical distance ($r = 0.36$, $P = 0.038$) therefore indicating limitations in gene flow. In the multiple regression analysis, variation in petal length (flower size) and presence of hairs correlated with the genetic distance between populations (Table 3). Populations of *A. urbicum* had the largest

flowers and usually no hairs on petals, sepals and ovaries, *A. hierrense* and *A. pseudourbicum* had intermediate-sized flowers with hairs and *A. appendiculatum* had the smallest flowers with no hairs (Table 1).

Of all the vegetative and reproductive characters, none were positively correlated with mean annual rainfall whereas growth form (module length), leaf form and the number of petals varied along a longitudinal gradient (Table 3). The study of latitudinal variation within Tenerife revealed a significant positive association with inflorescence length. Three morphological variables were negatively associated with the predictor variables. Populations appeared to be similar in leaf form when their habitats were most different in mean annual rainfall and had similar filament length or petal width when they were most distant in longitude or latitude, respectively.

DISCUSSION

Considerable variation was present in all vegetative and reproductive traits across populations of the four species considered together. Only two variables, both related to flower morphology, correlated with the genetic markers. Phylogeny may hence be of minor importance in explaining morphological variation. Ecological factors are more likely predictors of variance in a number of traits. While differences in rainfall between localities appear to have no significant positive effect on the morphological characters measured, both the latitudinal and longitudinal position of



	urbicum S. Marcos	urbicum El Sauzal	urbicum Anaga	urbicum Guimar	urbicum El Rio	appendiculatum Alajeró	pseudourbicum Escalona	pseudourbicum Santiago del Teide	pseudourbicum Masca	hierrense Gallegos	hierrense Valverde	hierrense El Golfo
Differences to <i>A. canariense</i> :												
minimum no. of AFLPs	33	25	25	28	28	19	30	31	26	28	29	28
maximum no. of AFLPs	36	31	31	32	34	27	36	36	29	31	32	32

Figure 3. Neighbour-joining tree of *Aeonium* sect. *Leuconium* based on Nei & Li's (1979) genetic distance. The populations of the four species in the present investigation are in bold. The table gives the maximum and minimum number of differences in AFLPs between each of the 12 populations and the outgroup *A. canariense*. The distribution of species on islands is indicated (G = La Gomera, GC = Gran Canaria, H = Hierro, L = Lanzarote, P = La Palma, T = Tenerife). Bootstrap values above 50% are indicated above branches (1000 bootstraps).

Table 3. Multiple regression of morphological variables on four matrix variables in 12 populations of *A. urbicum*, *A. appendiculatum*, *A. hierrense* and *A. pseudourbicum*. Backwise elimination procedure was used. SPRC = standard partial regression coefficients. Effects were found significant in each step after 999 permutations when probabilities were smaller than a Bonferroni-corrected $\alpha = 0.05$. Only significant effects are shown. Regressions were carried out separately on latitude for Tenerife populations only

Character	Genetics		Rainfall		Longitude		Model		Latitude		Model	
	SPRC	P	SPRC	P	SPRC	P	R ²	P	SPRC	P	R ²	P
GROWTH FORM												
Module length	–	–	–	–	0.58	0.002	0.34	0.002	–	–	–	–
Flowering probability	–	–	–	–	–	–	–	–	–	–	–	–
LEAF FORM	–0.16	0.001	0.85	0.001	0.74	0.001	–	–	–	–	–	–
FLOWER MORPHOLOGY												
Hair	0.54	0.001	–	–	–	–	0.29	0.001	–	–	–	–
Petal length	0.36	0.019	–	–	–	–	0.13	0.021	–	–	–	–
Petal width	–	–	–	–	–	–	–	–	–0.36	0.049	0.13	0.019
Sepal length	–	–	–	–	–	–	–	–	–	–	–	–
Filament length (inner whorl)	–	–	–	–	–0.25	0.011	0.06	0.107	–	–	–	–
Filament length (outer whorl)	–	–	–	–	–	–	–	–	–	–	–	–
Nectary length	–	–	–	–	–	–	–	–	–	–	–	–
Nectary width	–	–	–	–	–	–	–	–	–	–	–	–
Ovary length	–	–	–	–	–	–	–	–	–	–	–	–
Ovary width	–	–	–	–	–	–	–	–	–	–	–	–
Style length	–	–	–	–	–	–	–	–	–	–	–	–
Number of petals	–	–	–	–	0.44	0.021	0.20	0.021	–	–	–	–
INFLORESCENCE												
Length	–	–	–	–	–	–	–	–	0.50	0.001	0.25	0.019
Width	–	–	–	–	–	–	–	–	–	–	–	–

a population are associated with the morphological variation in the 12 populations. An explanation for this association can only be suggestive due to the undefined nature of these two environmental variables. Increasing longitude was associated with shorter modules and a shift from oblanceolate to oblongate leaves as well as a decrease in the mean number of petals. Shorter modules and oblongate leaves (i.e. shorter leaves) may reflect responses to less optimal conditions for growth and stem extension the more oceanic the climate. Less optimal conditions may take the form of less solar radiation and more competition among plants in the more lush habitats of the western islands. The number of petals in a flower is identical to the number of fruits and half the number of stamens and may therefore be genetically correlated with the male and female fitness of the flower. The present results indicate that the patterns of sex allocation vary along the longitudinal gradient as the number of petals changes, but more components of reproduction must be included to confirm this. The

north–south gradient in inflorescence length revealed by the significant effect of latitude on this variable may reflect responses to less optimal conditions in the south (e.g. higher temperatures).

Three morphological traits correlated negatively with ecological factors. Unless this is due to a negative association between distance in the ecological factors used in the present investigation and distance in unidentified ecological factors of importance to these traits, this pattern has no apparent evolutionary or ecological explanation and may best be interpreted as an artifact from the sample design.

The genus *Aeonium* has been invoked as a prime example of adaptive radiation in plants (Lems, 1960; Voggenreiter, 1974). Its species richness, the restricted distribution of most of its members to specific habitats and the diversity of growth forms exhibited by the genus support this argument. The present results confirm that module length, which is one of the main components of growth form, as well as leaf morphology, inflorescence length and the number of petals in

a flower are responsive to the varying ecological conditions of the Canary Islands and may be interpreted as adaptive. To fully confirm these conclusions, the heritability of these traits must be investigated just as a more direct demonstration of current selection in natural populations of *Aeonium* is needed.

Aeonium appendiculatum was formerly regarded as a slightly divergent form of *A. urbicum* of Tenerife (Liu, 1989) and has only recently been named as a separate species (Bañares Baudet, 1999). The obtained AFLPs in the present investigation clearly suggest that the monocarpic *Aeonium* of La Gomera is genetically divergent from other species in the section and therefore support its taxonomic separation. While populations of *A. urbicum* and populations of *A. hierrense* appear genetically similar within species groups, the population of *A. pseudourbicum* in the isolated Barranco of Masca differs remarkably from its conspecifics. A genetic similarity to *A. hierrense* is indicative of either introgression or a common ancestral relationship. A more extensive phylogenetic investigation of the species is needed to elucidate this. No final conclusions about the internal relationships of the species in the section as a whole can be made due to the low support of the basal branches in the tree.

In the taxonomic literature, characters related to leaf form, pubescence of flowers and height of the individual plants are among those that distinguish *A. urbicum* and *A. appendiculatum* from the two other species (Liu, 1989; Bañares Baudet, 1992). *Aeonium pseudourbicum* is distinguishable from *A. hierrense* based on its leaf form (Bañares Baudet, 1992) whereas size of inflorescences and flower organs and the morphology of ovaries and styles distinguish *A. urbicum* from *A. appendiculatum* (Bañares Baudet, 1999). The nested ANOVAs on morphological variables confirm that species can be separated based on variation in leaf form, inflorescence width, pubescence of floral organs and flower size. Only the latter two variables, however, correlate with the variation in the genetic marker across all populations and may be long-term fixed traits. As species in this investigation are arranged roughly on a line of increasing longitude it is not surprising to find a significant separation of species based on a morphological character that also correlates with longitude, as is the case for leaf form.

It is surprising that a phylogenetic component is found in only two morphological traits in the four species. Previous analysis of growth forms within the genus revealed prominent differences in module length, branching and flowering probabilities and branching angles between species of different taxonomic sections or phylogenetic clades (Jorgensen & Olesen, 2000). The present results suggest that radiations and morphological differentiation at lower taxonomic levels in the genus may partly be the result of

responses to current ecological conditions and therefore not reflected in the phylogeny. The presence of morphological characters that have irregular patterns of variation not conforming to either ecological variables across populations or variation in the genetic markers may reflect stochasticity or an additional influence of past adaptations in the evolution of the four *Aeonium*-species. It is also possible that unexplored ecological factors, as for example soil factors and solar radiation, may be relevant to the determination of the variation in these traits.

Considerable variation within populations in both vegetative and reproductive traits is revealed by the R^2 of the growth models and size-corrected flower traits, the percent of correct predictions in the logistic regressions and the coefficients of variance for the remaining traits. Genetic variation in the population as well as the plants' exploration of resources in a mosaic microenvironment may explain this morphological variation. Analysis of growth forms in single populations of 22 species of *Aeonium* showed similar patterns of considerable intrahabitat variation (Jorgensen & Olesen, 2000). It is possible that the pronounced variation in morphological characters in *Aeonium* is due to a general high level of genetic variation in the species and populations investigated. Alternatively, it may be explained by the fact that species of *Aeonium* often occupy habitats with pronounced differences in microhabitats so that phenotypic plasticity in morphological characters becomes apparent. For example, large parts of the Canary Islands have temporal or permanent exposure to high solar irradiance and this may sort microhabitats into those offering shade and those that do not. In such a mosaic environment the possibility exists for plants to evolve morphological responses to their microhabitat (e.g. Turkington & Harper, 1979). The majority of habitats of *A. urbicum*, *A. appendiculatum*, *A. hierrense* and *A. pseudourbicum*, however, are disturbed areas, often abandoned fields, and probably do not offer the stability needed for the evolution of such microhabitat specializations. It is evident that a detailed experimental design is needed to test whether variation in morphological characters within a population is actually due to microhabitat variation and whether it can be ascribed to phenotypic plasticity in general or to fixed, microevolutionary responses.

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