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Evolution of *Dystaenia takesimana* (Apiaceae), endemic to Ullung Island, Korea

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Abstract. *Dystaenia* (Apiaceae) consists of two species, one distributed in Japan (*D. ibukiensis*), and the other endemic to Ullung Island, Korea (*D. takesimana*). In comparison with representative outgroup taxa in *Ligusticum*, *Seseli*, *Angelica*, and *Osmorhiza*, *Dystaenia* is shown to be monophyletic based on sequences from chloroplast *trn*L-F intron and spacer regions confirming previously published results using ITS sequences. Loss of one large part of *trn* L-F in *D. takesimana* strongly suggests that this species evolved from *D. ibukiensis* rather than the reverse. AFLP analysis within and among twelve populations (six from each species; total 126 individuals) using three primer combinations reveals 130 reliable fragments. Neighbour-joining analysis shows the two species to be distinct populational systems. Levels of overall genetic variation as measured by Shannon Diversity are significantly higher in *D. takesimana*. Geographic structuring of genetic variation occurs within *D. ibukiensis* but not within *D. takesimana*, suggesting that the Ullung species exists as a single population. It is hypothesised that after a founder-effect reduction of genetic variation, anagenetic speciation may have occurred in *D. takesimana* by gradual morphological divergence accompanied by accumulation of genetic variation through mutation, recombination and drift.

Key words: AFLP, anagenesis, *Dystaenia*, evolution, genetic diversity, island biology, Japan, *trn*L-F, Ullung Island.

Introduction

Oceanic islands are model systems for studying evolutionary patterns and processes (e.g. Emerson 2002, Grant 1998, Stuessy and Ono 1998). Features such as high levels of endemism, reduced gene flow due to oceanic barriers, and young geological ages make them attractive settings for studying evolution, especially in contrast to more complex mainland systems. Many investigations in recent years on processes of plant evolution and speciation on oceanic archipelagos have yielded important

insights, such as in the Hawaiian Islands Wagner and Funk 1995, Carlquist et al. 2003), the Canary Islands (Barber et al. 2000, Helfgott et al. 2000, Mort et al. 2002), the Galápagos Islands (Nielsen et al. 2003), the Juan Fernandez Islands (Crawford et al. 1998, Stuessy et al. 1998, Jensen et al. 2002), and the Bonin Islands (Ito et al. 1998).

Another oceanic island of interest is Ullung Island, located off the coast of South Korea in the Eastern (Japan) Sea (Fig. 1). Ullung is of volcanic origin and situated 137 km east of mainland Korea and c. 300 km west from Japan; the total area is approximately 73 km^2 and the highest peak is 984 m tall. The first eruptions under the sea occurred about 2.7 million years ago, and the maximum age of the island during which it would have been available for colonisation is about 1.8 million years (Kim 1985). The climate is oceanic with relatively small differences in temperature throughout the year (Kim 1988). Ullung has once been covered by dense primary forests before being cut down (Nakai 1928). The present vegetation, apart from coastal cliffs and cultivated areas, consists of secondary forest and small remnants of the original forest types (Kim 1985). The flora contains c. 650 vascular plant species of which about 500 are native or endemic (Lee and Yang 1981). About 37 angiosperm taxa are endemic, and it has been hypothesised that all of them have evolved from their continental progenitors by anagenesis (Sun and Stuessy 1998), i.e. by phyletic speciation from progenitors (Stuessy et al. 1990). There are no known cases of cladogenesis or conspicuous adaptive radiation seen so commonly in other more complex island systems such as Hawaii or the Galapagos Islands. To date, detailed evolutionary studies on the origin of Ullung endemics have centred

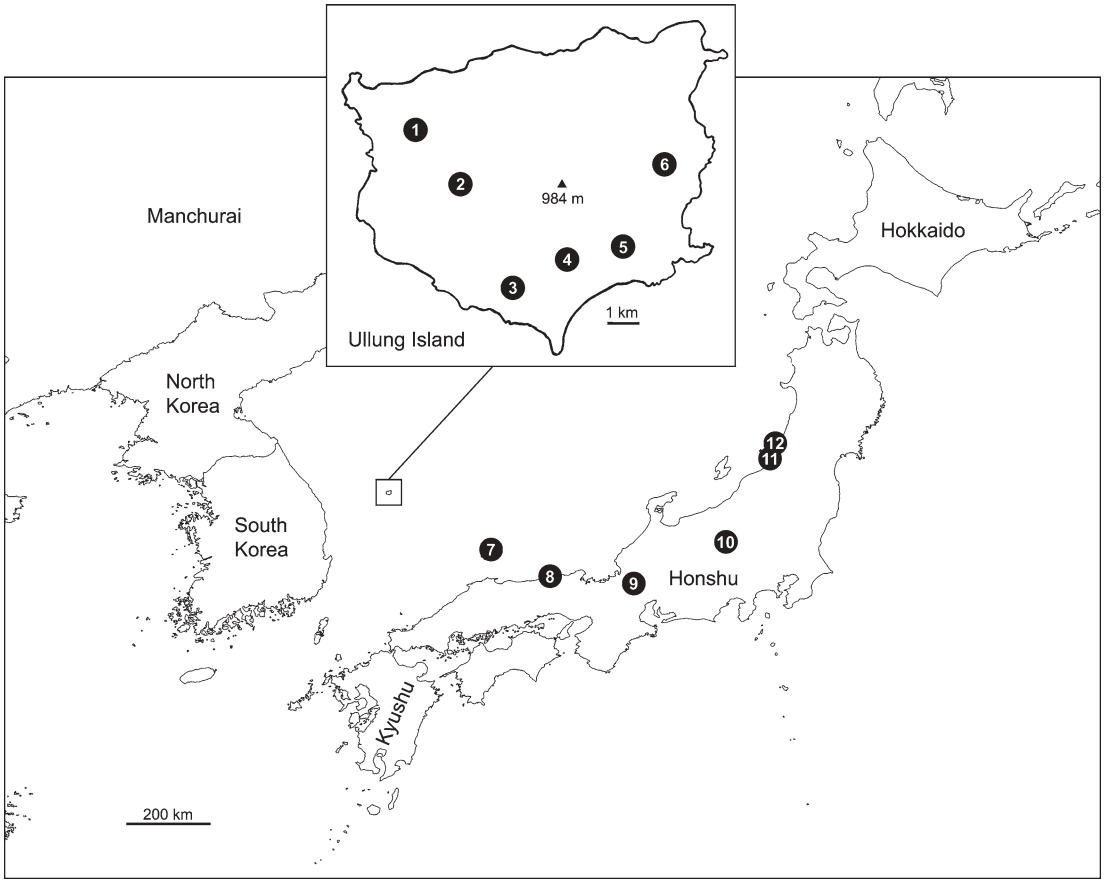

Fig. 1. Populations of *Dystaenia takesimana* (1–6) and *D. ibukiensis* (7–12) from Ullung Island and Japan

on *Acer* (Pfosser et al. 2002) and *Hepatica* (Pfosser et al. in press). This paper focuses on another genus, *Dystaenia* (Apiaceae).

Dystaenia contains only two species (Kitagawa 1937), *D. ibukiensis* endemic to Japan, and *D. takesimana* endemic to Ullung Island. Both species are morphologically very similar but do show differences (Suk et al. 1974, Sun et al. 1997). Sun et al. also showed that *D. takesimana* is larger in size, and that it expresses less variation in some morphological characters in comparison to the Japanese congener. The two taxa also have the same chromosome number, $2n = 44$, as reported by Arano and Saito (1980) and Sun and Stuessy (1998), but Weiss et al. (2002) showed presence of B-chromosomes in the former and their absence in the latter. Monophyly of *Dystaenia* and close relationship of the two species were also confirmed by a molecular study using ITS sequences (Choi et al. 1998).

The focus of our study is on the evolution of the island endemic *D. takesimana*. In a first step we tested the closeness of relationship between the two *Dystaenia* species. Another important question was if *D. ibukiensis* dispersed from Japan to Ullung and evolved into *D. takesimana* or vice versa. These questions were addressed using *trn*L-F intron and spacer sequences. The second step was to compare genetic variation within and between populations of both species to understand what changes have taken place during evolution. To analyse genetic variation we used amplified fragment length polymorphism (AFLP; Vos et al. 1995), a method successfully employed in many recent studies to reveal genetic variation on the populational level (e.g. Ishida et al. 2003, Schönswetter et al. 2003, Tremetsberger et al. 2003).

Materials and methods

Sampling. Six populations (62 individuals) of *Dystaenia takesimana* (Nakai) Kitagawa and six populations (64 individuals) of *D. ibukiensis* (Yabe) Kitagawa were sampled during 2000–2003 (Fig. 1, Table 1). From each population young leaves were collected from 9–15 individuals and stored in silica gel. Voucher specimens of all populations are deposited in JNU, MAK and WU (Table 1).

DNA extraction. Total genomic DNA was extracted from the dried leaves following the CTAB-protocol of Doyle and Doyle (1987) with slight modifications. The powdered leaf material, together with c. 5 mg PVP, was extracted in 700 μl CTAB buffer (2% CTAB, 100 mM Tris, 1.4 M NaCl, 20 mM EDTA, 0.2% mercaptoethanol, pH 8.0) for 30 min at 60°C.; 500 μl chloroform/isoamylalcohol (24:1) were added and the extraction mix was incubated for 15 min at 4°C. After centrifugation, the DNA was precipitated with 500 μl isopropanol. The pellet was washed with 70% ethanol and dissolved in 100 μl TE buffer.

AFLP fingerprinting. AFLP was performed according to the protocol provided with a commercial AFLP analysis kit (Invitrogen, U.S.A.) with minor modifications. 200 ng genomic DNA was digested with the restriction enzymes *Mse*I und *Eco*RI for three hrs in a total volume of 5 μl. T4-ligase and double-stranded adapters were added and the reaction was allowed to continue over night at 37°C. The ligated DNA fragments were diluted 10-fold and pre-amplified (20 cycles of 20 sec at 94°C, 40 sec at 56°C, 50 sec at 72°C). The PCR products were again diluted 10-fold and used for the selective amplification (20 sec at 94°C, 20 sec at 65°C, 50 sec at 72°C followed by 12 cycles with an annealing temperature decreasing by 0.7°C per cycle; during the remaining 23 cycles, annealing temperature was kept at 56°C) with different combinations of *Eco*RI and *Mse*I primers. All reactions were performed on a Thermo Hybaid Px2 thermal cycler (Thermo Electron Corporation, U.S.A.). An initial primer screening using 12 selective primer combinations was performed and three primer combinations giving clear and reproducible bands were selected for further analyses: *Eco*-ACT/*Mse*-CAC, *Eco*-ACC/*Mse*-CTG, *Eco*-AGG/*Mse*-CAT. The fluorescence-labelled selective amplification products were separated on a 5% polyacrylamide gel with an internal size standard (Genescan 500 Rox, PE Applied Biosystems) on an automated ABI 377 sequencer (Perkin Elmer, U.K.). The data were imported into Genographer (Version 1.6.0, Montana State University, http://hordeum.oscs.montana.edu/genographer) for scoring of the fragments; the results were exported as a presence/absence matrix for further analyses.

Table 1. Populations included in AFLP analyses. Abbreviations used: JNU, Chonbuk National University, MAK, Makino Herbarium, Tokyo Metropolitan University, WU, Institute of Botany, University of Vienna

Population	Origin	Elevation (m s. m.)	Voucher
1	Korea, Gun Prov. Ullung Island, Taeha-ri, Jung-ri	120	29.4.2001, T. Stuessy, B.-Y. Sun, M. Pfosser & T.-K. Paek 17549 (WU)
2	Korea, Gun Prov. Ullung Island, Taeha-ri, Taeharyong	470	29.4.2001, T. Stuessy, B.-Y. Sun, M. Pfosser & T.-K. Paek 17571 (WU)
3	Korea, Gun Prov. Ullung Island, Namyang-ri, Tonggumi	120	30.4.2001, T. Stuessy, B.-Y. Sun, M. Pfosser & T.-K. Paek 17601 (WU)
4	Korea, Gun Prov. Ullung Island, Sadong-ri	340	30.4.2001, T. Stuessy, B.-Y. Sun, M. Pfosser & T.-K. Paek 17607 (WU)
5	Korea, Gun Prov. Ullung Island, Dodong-ri, 1 km W of Kakidung	300	30.4.2001, T. Stuessy, B.-Y. Sun, M. Pfosser & T.-K. Paek 17620 (WU)
6	Korea, Gun Prov. Ullung Island, Naesujeon Mineral Water Fountain	100–210	28.4.2001, T. Stuessy, B.-Y. Sun, M. Pfosser & T.-K. Paek 17534 (WU)
7	Japan, Shimane Pref. Oki Co., Saigo Town, Igo to Nishimura, N36°19′38.9″, E133°17′04.3″	120	3.9.2002, H. Kato 020017 (MAK)
8	Japan, Hyogo Pref., Mikata Co. Hamasaka Town, Tainohama, N35°38′12.1″, E134°28′49.2″	20	2.9.2002, H. Kato 020010 (MAK)
9	Japan, Shiga Pref., Mt. Ibuki, limestone quarry company, N35°24′56.5″, E136°23′51.5″	900–940	2.6.2000, T. Stuessy, B.-Y. Sun & H. Kato 17335 (WU)
10	Japan, Nagano Pref., Komoro City, Hishino-onsen to Takamine-rindo, N36°21′37.0″, E138°27′32.3″	1080	21.8.2002, H. Kato & S. Katoh 020002 (MAK)
11	Japan, Niigata Pref. Murakami City Gedo, N38°14′09.8″, E139°28′15.7″	50	8.9.2002, H. Kato & S. Katoh 020035 (MAK)
12	Japan, Yamagata Pref., Nishitigawa Co. Atsumi Town, Koshizi-toge, N38°36′18.7″, E139°36′03.0″	150	8.9.2002, H. Kato & S. Katoh 020037 (MAK)

Sequencing of *trn*L-F. *trn*L-F intron and spacer sequences were obtained from one individual each of populations 1 to 6 of *Dystaenia takesimana* and one individual each of *D. ibukiensis* of populations 7 to 12. Sequences of the outgroup species of *Ligusticum officinale*, *Seseli hippomarathrum*, and *Angelica sylvestris*, were obtained from material grown in the Vienna Botanical Garden (garden accession numbers 103/2001, 40/2001, and 520/2001, respectively). These genera were determined as close relatives of *Dystaenia* from ITS studies (Choi et al. 1998). The sequence for *Osmorhiza berteroi* was obtained from GenBank (AF432006). This provides a more distant outgroup for comparison. PCR conditions and primers were the same as previously published (Pfosser et al. 2002). Amplified double-stranded DNA fragments were sequenced directly on an ABI 377 automated sequencer following the DYEnamicET cycle sequencing protocol (Amersham Pharmacia, Piscataway, N.J.). Both strands were sequenced and only sequences with less than 1% of missing data were recorded.

Data Analyses. DNA sequences have been aligned using the PileUp program of the GCG software package (Genetics Computer Group 1994). All sequences have been deposited in the EMBL

database (accession numbers AM109907 – AM109921). Phylogenetic analyses using neighbour-joining (NJ) and maximum parsimony (MP) were performed with the computer program PAUP* version 4.0b10 (Swofford 2003). Most parsimonious trees were obtained by 1000 replicates of random sequence addition using tree bisection-reconnection (TBR) branch swapping under the Fitch criterion (Fitch 1971). Ten thousand fast bootstrap replicates (Felsenstein 1985), were used to assess confidence limits for the resulting tree topologies. Indels in the data matrix were coded as additional characters, and tree searches were performed using the nucleotide data alone or together with the indel data. Tree manipulations to improve the appearance of trees (branch switching, labeling) were performed using MacClade version 3.06 (Maddison and Maddison 1992).

For each population number of AFLP fragments, number and percentage of polymorphic fragments, Shannon Diversity [$H_{Sh} = -\Sigma (p_j \ln p_j)$ where p_j is the relative frequency of the j-th fragment; Legendre and Legendre 1998], and the number of private fragments (fragments unique to a single population) were calculated. As additional diversity measures, mean number of pair-wise differences and average gene diversity over loci were calculated with Arlequin 2.0 (Schneider et al. 2000). Tests for significance of correlations and different mean values were calculated with SPSS 8.0 (SPSS Inc.). Analyses of molecular variance (AMOVAs) were calculated separate for both species with Arlequin 2.0 to partition the overall genetic variation into levels within and among populations.

A midpoint-rooted neighbour-joining phylogram based on Nei-Li distances for restriction-site data (Nei and Li 1979) was generated with PAUP* 4.0b10 (Swofford 2003). A principal coordinate analysis (PCoA) based on a matrix of inter-individual Jaccard distances ($1\text{-}C_J$, $C_J = a/a + b + c$, where a is the number of fragments shared between two individuals and b and c are the numbers unique to each individual) was calculated with the R package 4.0 d6 (Casgrain and Legendre 2001). The R package was also used to perform Mantel tests separate for each species (10,000 permutations): (a) the genetic matrix of inter-individual Jaccard distances was compared with a matrix of geographic distances between individuals in km, and (b) the goodness of fit of the genetic matrix and a model matrix of distance-classes was tested, whereby the geographical distances between individuals were coded in eight classes for *Dystaenia takesimana* and six classes for *D. ibukiensis*. Both analyses were also conducted with a genetic matrix of inter-populational F_{st} values obtained from Arlequin.

Pollen-ovule ratios were calculated to estimate the breeding system. Two flowers of each species were examined from herbarium specimens. Florets were dissected and anthers separated in 1% aceto-carmine, followed by heating over flame and observations by light microscopy. Total number of pollen grains per flower was counted. Pollen-ovule ratios were calculated as number of pollen grains from all five anthers divided by the number of ovules per flower (2).

Results

***trn*L-F sequences.** The aligned sequences yielded a data matrix of 984 bp in length. No DNA sequence variation was detected among populations within species (*D. ibukiensis* as well as *D. takesimana*). Between *D. ibukiensis* and *D. takesimana* a single A→G transition was found at position 223 of the aligned data matrix. In addition to this single transition all sequences of *D. takesimana* are characterised by a large deletion of 192 bp. This type of deletion is neither found in *D. ibukiensis* nor in any of the outgoup taxa and suggests that *D. takesimana* has evolved from *D. ibukiensis* rather than the reverse (Fig. 2). Similar tree topologies were obtained by NJ and MP analysis. Full heuristic search under MP criterion yielded a single most parsimonious tree both with or without inclusion of indels. A rather low bootstrap support for *D. takesimana* and *D. ibukiensis* (65%, Fig. 2) probably results from the large number of missing data due to the deletion in the sequences of *D. takesimana*. Additional indels are found in *Angelica sylvestris* and in the group *Angelica*, *Seseli*, *Ligusticum*, *Dystaenia* (Fig. 2). The latter two indels further support the close phylogenetic relationship among the four genera (the aligned data matrix with the position of all indels can be obtained from M.P. upon request).

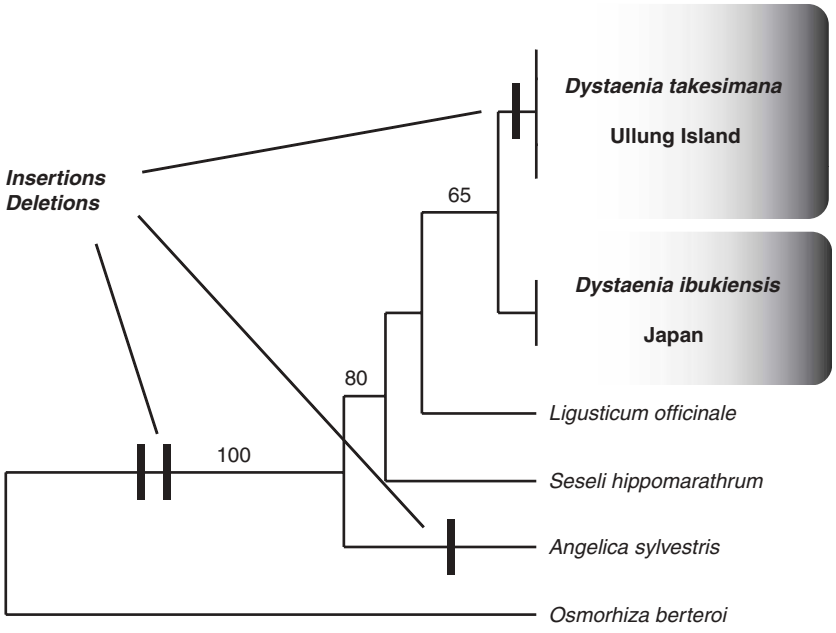

Fig. 2. Phylogenetic relationships based on neighbour-joining and maximum parsimony analysis of *trn*L-F sequences (bootstrap support values > 50% from maximum parsimony analysis are shown above branches). Correlation with the occurrence of a large deletion in *D. takesimana* is indicated

Genetic diversity within species and populations. From 126 individuals of both species a total of 130 unambiguously scorable fragments, all occurring in both species, were obtained; 100% are polymorphic. The length of the fragments varies from 56 to 400 bp. All 126 AFLP phenotypes are unique; private fragments are absent from all populations. In *Dystaenia takesimana* all 130 fragments are polymorphic. The number of fragments per population varies from 119 to 124, the percentage of polymorphic fragments from 93.33% to 98.39%, and H_{Sh} from 31.91 to 33.74 (Table 2). In *D. ibukiensis* 125 of the 130 fragments (96.15%) are polymorphic. The number of fragments per population varies from 115 to 124, the percentage of polymorphic fragments from 84.62% to 88.98%, and H_{Sh} from 27.04 to 30.48 (Table 2). The mean values for both species are given in Table 2. The number of fragments is not significantly different (t-test, $p = 0.18$), whereas the number of polymorphic fragments, the percentage of polymorphic fragments, and H_{Sh} are significantly higher in *D. takesimana* ($p < 0.001$). Mean number of pair-wise differences and average gene diversity over loci (not shown) confirm H_{Sh} and give no additional insights. H_{Sh} and estimated population size (Table 2) were tested for correlation separately for each species (Pearson, 2-tailed) and show a positive, but not significant, correlation (*D. takesimana*, $r^2 = 0.64$, $p = 0.054$; *D. ibukiensis*, $r^2 = 0.42$, $p = 0.17$).

Neighbour-joining analyses. The neighbour-joining phenogram (Fig. 3) among all individuals of both species shows a major separation between the species. Within *D. ibukiensis* the tree is further separated according to the populations with only a single individual of population 11 mixing with population 10. Population 12 is isolated from the other populations, although it is only 43 km away from population 11. In *D. takesimana* no separation of populations is visible. The PCoA (not shown) confirms these patterns.

AMOVAs, Mantel Test. Results of the two AMOVAs are given in Table 3. For *D.*

Table 2. Genetic diversity within populations of *Dystaenia takesimana* (1–6) and *D. ibukiensis* (7–12) and mean values of species (**significantly different with $p < 0.01$), standard deviation in parenthesis

Population	Sample size	Estimated population size	Number of fragments	Polymorphic fragments	Percentage of polymorphic fragments	Shannon Index
1	10	c. 100	120	112	93.33	31.91
2	10	> 500	124	122	98.39	33.51
3	10	> 500	123	118	95.93	33.17
4	11	> 1000	123	118	95.93	33.74
5	11	> 500	123	117	95.12	33.71
6	10	c. 100	119	117	98.32	32.76
Mean value			**122 (2)**	117.33** (3.20)	**96.17** (1.94)**	33.13** (0.70)
7	15	c. 100	124	110	88.71	30.48
8	10	< 50	115	98	85.22	27.93
9	10	< 50	117	99	84.62	27.04
10	9	< 50	119	103	86.55	29.42
11	10	< 50	124	107	86.29	29.88
12	10	c. 100	118	105	88.98	30.02
Mean value			**119 (3.73)**	103.67** (4.63)	86.73** (1.79)	29.13** (1.35)

takesimana 1.38% of the variation is found among populations and 98.62% within populations, for *D. ibukiensis* 18.71% of the variation is found among populations and 81.29% within populations. The R_M values calculated with inter-individual genetic and geographical distances are 0.042 ($p = 0.15$) for *D. takesimana* and 0.37 ($p = 0.0001$) for *D. ibukiensis*. The inter-populational approach gave similar results for *D. takesimana* ($R_M = -0.094$, $p = 0.39$), whereas for *D. ibukiensis* R_M was much lower and not significant ($R_M = 0.096$, $p = 0.32$). This different result might be due to autocorrelation among individuals within populations in the inter-individual approach. The Mantel test with distance classes using inter-individual genetic distances gave the following results: in *D. takesimana* only distance-class 1 (0 km) is significantly correlated ($R_M = 0.043$, $p = 0.01$), R_M for the other distance-classes ranges from −0.054 to 0.042 with all p-values > 0.1. In *D. ibukiensis* only distance-class 1 (0 km distance) shows a positive correlation ($R_M = 0.54$, $p = 0.001$), the other distance-classes show a weak to moderately negative correlation. The highest negative correlation is found among populations isolated by more than 500 km ($R_M = -0.19$, $p = 0.001$). Using inter-populational genetic distances (without a distance-class for 0 km), all R_M values for both species are low with $p > 0.1$.

Pollen-ovule ratio. The pollen-ovule ratio is 3788 for *Dystaenia ibukiensis* (two flowers: 3835,

Table 3. Analyses of molecular variance (AMOVA) for AFLP phenotypes in *Dystaenia takesimana* and *D. ibukiensis* (*$p < 0.05$, **$p < 0.01$)

Taxon	Source of variation	d. f.	Sum of squares	Variance components	Percentage of variation	F_{ST}
D. takesimana	Among populations	5	129.63	0.32	1.38	0.014*
	Within populations	56	1269.05	22.66	98.62	
D. ibukiensis	Among populations	5	342.40	4.58	18.71	0.19**
	Within populations	58	1155.06	19.91	81.29	

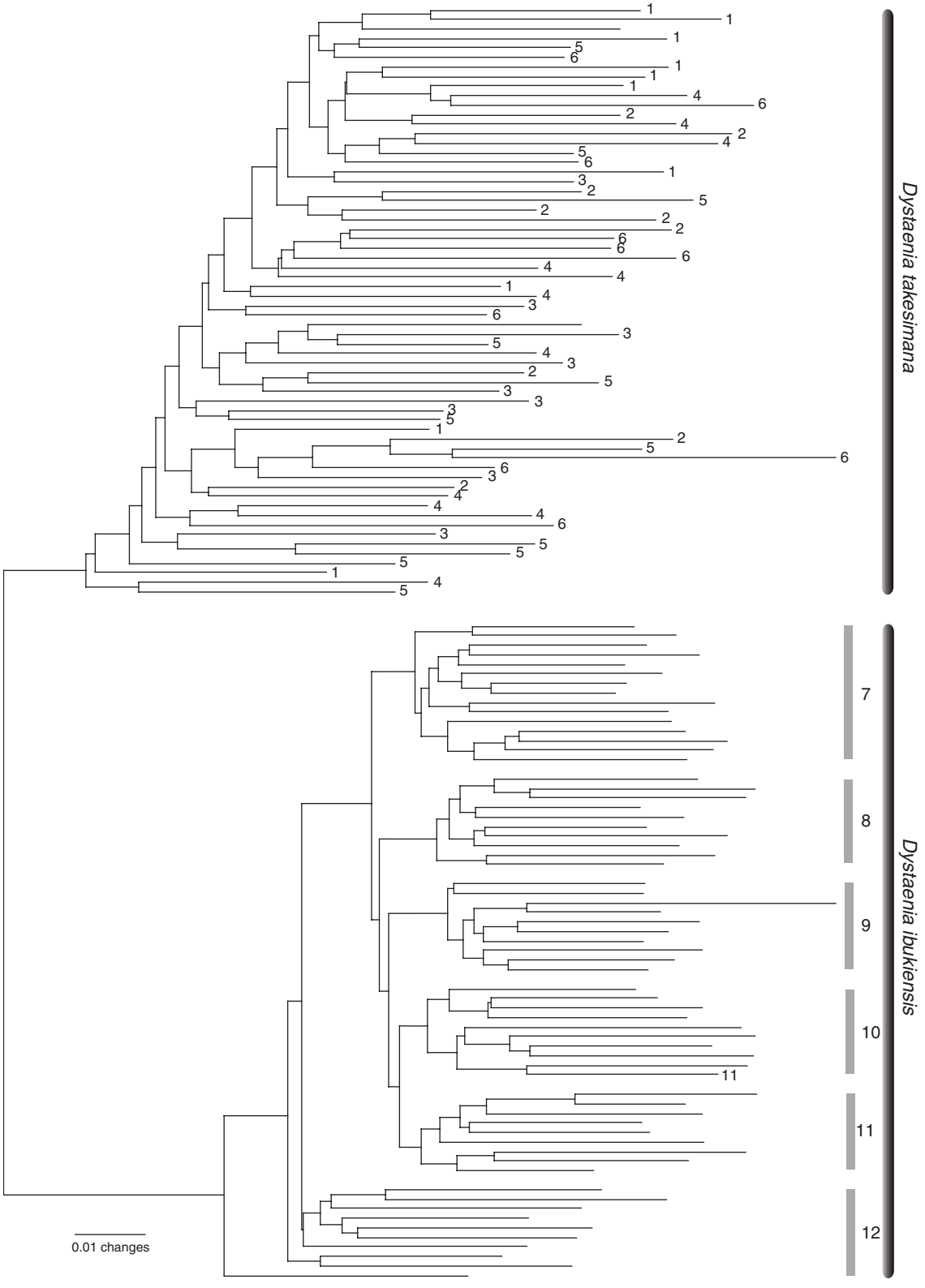

Fig. 3. Midpoint-rooted AFLP phenogram of a neighbour-joining analysis of pairwise Nei-Li distances among individuals of *Dystaenia takesimana* and *D. ibukiensis*. Numbers refer to populations (see Fig. 1 and Table 1)

3740) and 3875 for *D. takesimana* (3900, 3850), both falling within the averages for outcrossing species (Cruden 1977, 2000).

Discussion

Direction of evolution in *Dystaenia*. Our first aim was to ascertain whether *Dystaenia ibukiensis* from Japan dispersed to Ullung and gave rise to *D. takesimana* or the reverse. Ignoring more complex hypotheses involving extinction of unknown taxa, or simultaneous origin of both species from an unknown ancestor in an unknown land, these are the most probable hypotheses in this monophyletic genus of only two species. Further, because Ullung Island is geologically very young (1.8 my; Kim 1985), it is likely that the island endemic originated from that in Japan, rather than the reverse.

Analyses of *trn*L-F sequences (Fig. 2) provide helpful data bearing on evolutionary origins. A deletion found in the *trn*L-F region of *D. takesimana*, but not in *D. ibukiensis* and outgroup taxa, is most easily explained by the origin of the former from the latter. The other alternative, that the indel was first lost and then regained by *D. ibukiensis*, is by far less likely. It is most probable, therefore, that *D. ibukiensis* is the progenitor of *D. takesimana*. This result provides an important basis for the interpretation of genetic diversity within both species. It also supports, together with AFLP data showing no major subdivisions within *D. takesimana* (Fig. 3), the hypothesis of a single introduction of *D. ibukiensis* to Ullung. For interpretation of genetic diversity within both species, it is important to know their breeding systems. The indirect measure of pollen-ovule ratios of 3788 for *D. ibukiensis* and 3875 for *D. takesimana* are relatively high and both characteristic of xenogamous plants (Cruden 1977, 2000).

Phylogeographic patterns. The neighbour-joining dendrogram of the AFLP data (Fig. 3) shows a major subdivision between the two species, confirming results from sequence analyses that they are genetically distinct taxa. Within *D. ibukiensis*, the populations that are isolated by 43–613 km are genetically separated from each other. The relationships of the populations follow mainly their geographical dispositions. The only exception is population 12, which is strongly separated from the other populations although it is only 43 km away from population 11. The divergence between populations 11 and 12, which are geographically close, might be explained in part by the small population sizes and drift through time.

The relatively low percentage of variation among populations (18.71), in *D. ibukiensis*, however, and the lack of private fragments together with the low R_M of 0.37 in the inter-individual approach and 0.096 based on inter-populational distances, indicate considerable gene flow in spite of the relatively large geographic distances between the populations. Also the results of the Mantel test with distance classes, especially the inter-populational approach, support this hypothesis. The situation in *D. ibukiensis* contrasts with that in *Ranunculus glacialis* (Ranunculaceae), a presumably outcrossing species with comparable distances between populations (Schönswetter et al. 2004), in which clear evidence was found for isolation by distance. In this species R_M was 0.76, and the Mantel test with distance classes revealed significantly positive correlations in lower distance classes and significantly negative correlations among populations isolated by larger distances. It has to be noted, however, that *R. glacialis* is an alpine species that has undergone dramatic postglacial alterations and therefore not completely comparable.

The current geographic isolation of the populations of *D. ibukiensis* would argue against present large-scale gene flow among them. Habitat reduction in Japan in recent decades due to human activities could have interrupted a previously continuous distribution incorporating higher gene flow. Also, some of these localities occur on disturbed roadsides, often mowed or cut-over, which suggests the possibility that propagules could have been introduced inadvertently from other regions causing lack of correlation between genetic and geographic distances. This could also explain the isolated position of population 12 in the

neighbour-joining dendrogram. In *Dystaenia takesimana* no subdivisions are shown in the neighbour-joining tree; the individuals of all populations are mixed. This is in accordance with the low R_M resulting from the Mantel test.

Genetic diversity within species and populations. Outstanding features of both species of *Dystaenia* are high genetic diversity, as expressed by H_{Sh}, and the high percentage of polymorphic fragments (Table 2). All AFLP phenotypes are unique, which confirms our interpretation of outcrossing based on the high pollen-ovule ratios. Such high values are not expected in inbreeding species. That the populations of *D. takesimana* are not separated in the neighbour-joining tree (Fig. 3) and that variation among populations is low (low $F_{ST;}$ Table 3, plus low R_M), clearly show that the level of genetic exchange is very high and populations 1–6 are, in fact, samples of a single population consisting of all individuals of the species. The largest distance between two populations (1 and 6) was 8.1 km, which is not far from the highest possible distance on the small island; all individuals of the species apparently form a panmictic unit. This may also partly explain why measures of genetic diversity (Table 2) are higher than in *D. ibukiensis* where samples were taken from small and geographically isolated populations, since larger populations generally contain higher genetic diversity than do smaller ones (Loveless and Hamrick 1984, Barrett and Kohn 1991). Furthermore, even the sampled subpopulations of *D. takesimana* were larger than the populations of *D. ibukiensis* (Table 2).

Due to the founder effect, one would expect lower genetic diversity in the island species, at least in an early stage after colonisation. We assume, therefore, that *D. takesimana* regained genetic diversity during or after speciation. The maximum time of colonisation of *D. ibukiensis* is 1.8 million years (Kim 1985), the age of the island. The species could, of course, be much younger. It is also surprising that all fragments of the progenitor species are found in the island endemic. The founder population must have lacked some of the fragments found in the progenitor, *D. ibukiensis*. It might be hypothesised that after genetic reduction during the founding event, populations increased in size and distributed over the small island, accumulating higher levels of genetic diversity through mutation, recombination, and drift in the absence of strong selection via cladogenesis and adaptive radiation.

The higher genetic diversity in *D. takesimana* compared to *D. ibukiensis* contrasts with the results of Sun et al. (1997) on variation in leaf morphology. *Dystaenia takesimana* shows very limited range compared to *D. ibukiensis*, which was interpreted by the authors as a loss of variation due to the founder effect. Another possible explanation could be that the morphological variation in both genera is due, at least in part, to environmental canalisation rather than an expression of genetic diversity, the plants from Ullung being less variable because of the more uniform environment on the small island.

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References

Arano H., Saito H. (1980) Cytological studies in the family Umbelliferae. II. Karyotypes of some genera of *Bupleurum*, *Spuriopimpinella*, and *Pimpinella*. Kromosoma 2: 178–185.

Barber J. C., Francisco-Ortega J., Santos-Guerra A., Marrero A., Jansen R. K. (2000) Evolution of endemic *Sideritis* (Lamiaceae) in Macaronesia: insights from a chloroplast DNA restriction site analysis. Syst. Bot. 25: 633.647.

Barrett S. C. H., Kohn J. R. (1991) Genetic and evolutionary consequences of small population sizes in plants: implications for conservation. In: Falk D. A., Holsinger K. E. (eds.) Genetics and conservation of rare plants. Oxford University Press, New York, pp. 3–30.

Carlquist S., Baldwin B. G., Carr G. D. (eds.) (2003) Tarweeds & silverswords: evolution of the Madiinae (Asteraceae). Missouri Bot. Gard. Press, St. Louis.

Casgrain P., Legendre P. (2001) The R package for multivariate and spatial analyses, version 4.0 d6 – Users Manual. Département de Sciences Biologiques, Université de Montréal.

Choi H. -K., Kim Y., Sun B. -Y., Shin H. (1998) Phylogeny of *Dystaenia* in subfamily Apioideae (Family Apiaceae) based on ITS sequences. Kor. J. Plant Tax. 28: 139–149.

Crawford D. J., Sang T., Stuessy T. F., Kim S. -C., Silva O. M. (1998) *Dendroseris* (Asteraceae: Lactuceae) and *Robinsonia* (Asteraceae: Senecioneae) on the Juan Fernandez Islands: similarities and differences in biology and phylogeny. In: Stuessy T. F., Ono M. (eds.) Evolution and speciation of island plants. Cambridge University Press, Cambridge, pp. 97–120.

Cruden R. W. (1977) Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. Evolution 31: 32–46.

Cruden R. W. (2000) Pollen grains: why so many? Pl. Syst. Evol. 222: 143–165.

Doyle J. J., Doyle J. L. (1987) A rapid DNA isolation procedure for small amounts of fresh leaf tissue. Phytochem. Bull. 19: 11–15.

Emerson B. C. (2002) Evolution on oceanic islands: molecular phylogenetic approaches to understanding pattern and process. Molec. Ecol. 11: 951–966.

Felsenstein J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791

Fitch W. M. (1971) Toward defining the course of evolution: minimum change for a specific tree topology. Syst. Zool. 20: 406–416.

Genetics Computer Group (1994) Program manual for the Wisconsin package, Version 8. GCG, Madison, Wisconsin, USA.

Grant P. R. (ed.) (1998) Evolution on islands. Oxford University Press, Oxford.

Helfgott D. M., Francisco-Ortega J., Jansen R. K., Simpson B. B. (2000) Biogeography and breeding system evolution of the woody *Bencomia* alliance (Rosaceae) in Macaronesia based on ITS sequence data. Syst. Bot. 25: 82–97.

Ishida T. A., Hattori K., Sato H., Kimura M. T. (2003) Differentiation and hybridization between *Quercus crispula* and *Q. dentata* (Fagaceae): insights from morphological traits, amplified fragment length polymorphism markers, and leaf miner composition. Amer. J. Bot. 90: 769–776.

Ito M., Soejima A., Ono M. (1998) Genetic diversity of the endemic plants of Bonin (Ogasawara) Islands. In: Stuessy T. F., Ono M. (eds.) Evolution and speciation of island plants. Cambridge University Press, Cambridge, pp. 141–154.

Jensen R. J., Schwoyer M., Crawford D. J., Stuessy T. F., Anderson G. J., Baeza C. M., Silva O. M., Ruiz E. (2002) Patterns of morphological and genetic variation among populations of *Myrceugenia fernandeziana* (Myrtaceae) on Masatierra Island: implications for conservation. Syst. Bot. 27: 534–547.

Kim Y. K. (1985) Petrology of Ulreung volcanic island, Korea. 1. Geology. J. Jap. Assoc. Mineral., Petrol. & Econ. Geol. 80: 128–135.

Kim J. -W. (1988) The phytosociology of forest vegetation on Ulreung-do, Korea. Phytocoenologia 16: 259–281.

Kitagawa M. (1937) Miscellaneous notes on Apiaceae (Umbelliferae) of Japan and Manchurai. II. Bot. Mag. (Tokyo) 51: 805–812.

Lee W. T., Yang I. S. (1981) The flora of Ulreung and Dogo Island. In: Anon (eds.) A Report on the Scientific Survey of the Ulreung and Dogdo Islands. The Korean Association for Conservation of Nature, Seoul, pp. 61–95.

Legendre P., Legendre L. (1998) Numerical ecology. Elsevier, Amsterdam.

Loveless M. D., Hamrick J. L. (1984) Ecological determinants of genetic structure in plant populations. Annual Rev. Ecol. Syst. 15: 65–95.

Maddison W. P., Maddison D. R. (1992) MacClade: Analysis of phylogeny and character evolution. Version 3.0. Sinauer Associates, Sunderland, Massachusetts.

Mort M. E., Soltis D. E., Soltis P. S., Francisco-Ortega J., Santos-Guerra A. (2002) Phylogenetics and evolution of the Macaronesian clade of Crassulaceae inferred from nuclear and chloroplast sequence data. Syst. Bot. 27: 271–288.

Nakai T. (1928) The vegetation of Dagelet Island: Its formation and floral relationship with Korea and Japan. Proc. 3rd Pan-Pacific Sci. Congr. 1: 911–914. Tokyo.

Nei M., Li W. H. (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76: 5269–5273.

Nielsen L. R., Cowan R. S., Siegismund H. R., Adsersen H., Philipp M., Fay M. F. (2003) Morphometric, AFLP and plastid microsatellite

variation in populations of *Scalesia divisa* and *S. incisa* (Asteraceae) from the Galapagos Islands. Bot. J. Linn. Soc. 143: 243–254.

Pfosser M. F., Guzy-Wróbelska J., Sun B. -Y., Stuessy T. F., Sugawara T., Fujii N. (2002) The origin of species of *Acer* (Sapindaceae) endemic to Ullung Island, Korea. Syst. Bot. 27: 351–367.

Pfosser M., Sun B. -Y., Stuessy T. F., Jang C. -G., Guo Y. -P., Kim T., Hwan K. C., Kato H., Sugawara T. (2005) Phylogeny of *Hepatica* (Ranunculaceae) and origin of *Hepatica maxima* Nakai endemic to Ullung Island, Korea. Biol. J. Linn. Soc. (in press)

Schneider S., Roessli D., Excoffier L. (2000) Arlequin ver. 2.000: A software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Schönswetter P., Tribsch A., Schneeweiss G. M., Niklfeld H. (2003) Disjunctions in relict alpine plants: phylogeography of *Androsace brevis* and *A. wulfeniana* (Primulaceae). Bot. J. Linn. Soc. 141: 437–446.

Schönswetter P., Tribsch A., Niklfeld H. (2004) Glacial history of high alpine *Ranunculus glacialis* (Ranunculaceae) in the European Alps in a comparative phylogeographical context. Biol. J. Linn. Soc. 81: 183–195.

Stuessy T. F., Crawford D. J., Marticorena C. (1990) Patterns of phylogeny in the endemic vascular flora of the Juan Fernandez Islands, Chile. Syst. Bot. 15: 338–346.

Stuessy T. F., Crawford D. J., Marticorena C., Silva O. M. (1998) Isolating mechanisms and modes of speciation in endemic angiosperms of the Juan Fernandez Islands. In: Stuessy T. F., Ono M. (eds.) Evolution and speciation of island plants. Cambridge University Press, Cambridge, pp. 79–98.

Stuessy T. F., Ono M. (eds.) (1998) Evolution and speciation of island plants. Cambridge University Press, Cambridge.

Suk K. -T., Nitta A., Konoshima M. (1974) Studies on origin of Japanese Chuanxiong. III. *Dystaenia*. Yakugaku Zasshi 94: 1251–1256.

Sun B. -Y., Park J. H., Kim C. H., Kim K. S. (1997) Morphological divergence of the genus *Dystaenia* (Apiaceae). Kor. J. Plant Tax. 27: 481–490.

Sun B. -Y., Stuessy T. F. (1998) Preliminary observations on the evolution of endemic angiosperms of Ullung Island, Korea. In: Stuessy T. F., Ono M. (eds.) Evolution and speciation of island plants. Cambridge University Press, Cambridge, pp. 181–202.

Swofford D. L. (2003) PAUP*. Phylogenetic analyses using parsimony (* and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Tremetsberger K., Stuessy T. F., Guo Y. -P., Baeza C. M., Weiss H., Samuel R. M. (2003) Amplified Fragment Length Polymorphism (AFLP) variation within and among populations of *Hypochaeris acaulis* (Asteraceae) of Andean southern South America. Taxon 52: 237–245.

Vos P., Hogers R., Bleeker M., Reijans M., van de Lee T., Hornes M., Frijtes A., Pot J., Peleman J., Kuiper M., Zabeau M. (1995) AFLP: a new technique for DNA fingerprinting. Nucl. Acids Res. 23: 4407–4414.

Wagner W. L., Funk V. A. (eds.) (1995) Hawaiian biogeography: evolution on a hot spot archipelago. Smithsonian Institution, Washington, D.C.

Weiss H., Sun B. -Y., Stuessy T. F., Kim C. H., Kato H., Wakabayashi M. (2002) Karyology of plant species endemic to Ullung Island (Korea) and selected relatives in peninsular Korea and Japan. Bot. J. Linn. Soc. 138: 93–105.

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