Genetic Consequences of Anagenetic Speciation in Endemic Angiosperms of Ullung Island, Korea

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Two major modes of speciation exist in the evolution of endemic plants of oceanic islands: cladogenesis and anagenesis. The former is where an immigrant population becomes established and then disperses into different ecological zones on the same, or neighboring, island. Over time and in isolation, these different lineages diverge and adapt to their new environments, such that eventually they are regarded as distinct species. These cladogenetic radiations have been chronicled in considerable detail in Hawaii, Galápagos, and the Canary Islands. Anagenesis is a process of transformation of species. After establishment, an immigrant population grows in size but does not split into divergent lines due to a lack of ecological opportunity. Gene flow is maintained among subpopulations, and genetic variation accumulates due to mutation and recombination. The highest known level of anagenesis in the endemic flora of any oceanic island occurs in Ullung Island, Korea. This small island lies 137 km east of the Korean Peninsula. It is of volcanic origin, with no known connections to the mainland. Here, ca. 33 endemic species occur that represent transformational evolution from original immigrants during the past not more than 1.8 million years. A number of studies in this island have been done on isozymic genetic variation in natural populations and more recently also using AFLP and SSR molecular markers. The results from isozymes show a pattern of very limited genetic variation within these endemic species. More comprehensive AFLP and SSR markers, however, reveal much higher levels of variation in some species, even to the extent of approximating that seen in progenitor populations on the continent. The studies in Ullung Island show that anagenetic species can be expected to harbor high levels of genetic variation similar to that of the progenitor population. The individuals in subpopulations on the island appear to be held together genetically by gene flow such that no geographic partitioning of the genetic variation occurs over the landscape.

**Key words:** AFLP, evolution, isozymes, population genetics, SSR, Ullung-do.

It has long been recognized that oceanic archipelagos provide opportunities for understanding patterns and processes of plant evolution (Carlquist 1974, Whittaker and Ferrández-Palacios 2009, Bramwell and Caujapé-Castells 2011). Islands are especially
attractive as study sites of the origin of plant diversity because they are surrounded by water and hence isolated from other land masses, often hundreds or thousands of kilometers distant. It is compelling to ask questions as to how the colonizers arrived to the islands, from where they came, and how they proliferated and diversified after arrival.

Among the evolutionary processes of interest in plants of islands is speciation. Studies have shown numerous different modes of speciation in continental regions, such as allopatric speciation, peripatric speciation, allopolyploidy, autopolyploidy, catastrophic speciation, and progenitor-derivative speciation, among others (Grant 1981, Crawford 2010). In oceanic islands, the options appear to be fewer, especially due to a near absence of mechanisms involving chromosome change during speciation (Carr 1998, Stuessy and Crawford 1998, Weiss et al. 2002).

As might be expected, many investigators have focused on speciation in large endemic groups of the Hawaiian Islands, the Galápagos Islands, and the Canary Islands. Hawaii has particularly received much attention due to its high level of endemism at the specific level (89%, Wagner et al. 1999). Perhaps most spectacular has been the evolution of the lobelioid complex (Campanulaceae), encompassing some 110 woody endemic species (Wagner et al. 1999), all apparently from a single founding event (Givnish et al. 2009). In the Canary Islands, genera such as Echium (Boraginaceae; Böhle et al. 1996), Dendroseronchus (Asteraceae; Kim et al. 1996a, b), and Aeonium (Crassulaceae; Mort et al. 2002, Jorgensen and Olesen 2001) have been intensively studied. In the Galápagos Islands, the largest genus Scalesea (Asteraceae) has also been examined (Eliasson 1974, Schilling et al. 1994, Nielsen 2004).

Endemic plants of smaller oceanic archipelagos, however, have also been investigated for processes of speciation. Important in this regard are the Juan Fernández Archipelago and the Bonin (Ogasawara) Islands. In the former, speciation has been studied in the largest genera (all Asteraceae), Dendroseris (Sanders et al. 1987, Sang et al. 1994), Robinsonia (Sanders et al. 1987, Crawford et al. 1993a, Sang et al. 1995, Takayama et al. 2015a), and Erigeron (Valdebenito et al. 1992, López-Sepúlveda et al. 2015). Likewise, in the Bonin Islands, speciation has been examined in Crepidiastrum (Ito and Ono 1990), Ilex (Setogouchi and Watanabe 2000), and others.

In nearly all of the above examples, the principal type of speciation examined has been allopatric, whereby individuals from the founding lineage disperse into different habitats and adapt to new environments. Usually the morphology of each lineage transforms to such an extent that a new species is recognized. This process, therefore, is one of splitting of the original immigrant population and hence has been referred to as cladogenesis (Rensch 1947, 1960, Grant 1985). It is also an evolutionary radiation based on rapid adaptation to new environments, and hence, it is commonly also known as adaptive radiation (Schluter 2000, Givnish 2015).

But despite these examples of groups in oceanic islands originating by adaptive radiation, this does not explain the origin of all endemic
Table 1. Features of island systems, number of endemic species, and levels of anagenetic vs. cladogenetic speciation. Modified from Stuessy et al. (2006)

<table>
<thead>
<tr>
<th>Island system</th>
<th>Number of islands</th>
<th>Size (km²)</th>
<th>Distance from mainland (km)</th>
<th>Age (Myo)</th>
<th>Elevation (m)</th>
<th>Number of endemic species</th>
<th>Anagenetic speciation (%)</th>
<th>Cladogenetic speciation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hawaii</td>
<td>8</td>
<td>16885</td>
<td>3660</td>
<td>5</td>
<td>4250</td>
<td>828</td>
<td>7</td>
<td>93</td>
</tr>
<tr>
<td>Canary</td>
<td>7</td>
<td>7601</td>
<td>100</td>
<td>21</td>
<td>3710</td>
<td>429</td>
<td>16</td>
<td>84</td>
</tr>
<tr>
<td>Tristan da Cuhna</td>
<td>4</td>
<td>208</td>
<td>2580</td>
<td>18</td>
<td>2060</td>
<td>27</td>
<td>33</td>
<td>67</td>
</tr>
<tr>
<td>Juan Fernández</td>
<td>3</td>
<td>100</td>
<td>600</td>
<td>4</td>
<td>1319</td>
<td>97</td>
<td>36</td>
<td>64</td>
</tr>
<tr>
<td>Cape Verde</td>
<td>12</td>
<td>4033</td>
<td>570</td>
<td>10</td>
<td>2829</td>
<td>68</td>
<td>37</td>
<td>63</td>
</tr>
<tr>
<td>Galápagos</td>
<td>16</td>
<td>7847</td>
<td>930</td>
<td>5</td>
<td>1707</td>
<td>133</td>
<td>43</td>
<td>57</td>
</tr>
<tr>
<td>Madeira</td>
<td>3</td>
<td>792</td>
<td>630</td>
<td>14</td>
<td>1862</td>
<td>96</td>
<td>48</td>
<td>52</td>
</tr>
<tr>
<td>Ogasawara</td>
<td>12</td>
<td>99</td>
<td>800</td>
<td>Tertiary</td>
<td>916</td>
<td>118</td>
<td>53</td>
<td>47</td>
</tr>
<tr>
<td>St Helena</td>
<td>1</td>
<td>123</td>
<td>1850</td>
<td>15</td>
<td>826</td>
<td>36</td>
<td>53</td>
<td>47</td>
</tr>
<tr>
<td>Ullung</td>
<td>1</td>
<td>73</td>
<td>130</td>
<td>2</td>
<td>984</td>
<td>37</td>
<td>88</td>
<td>12</td>
</tr>
</tbody>
</table>

species. Some immigrants never split and diverge adaptively. Instead, after arrival they increase in population size, accumulate genetic diversity through mutation and recombination, and slowly transform morphologically into what is recognized as a new species distinct from continental progenitors. Such a process is gradual and has been called anagenesis (Stuessy et al. 2006).

To date, the oceanic island that has shown the highest level of anagenesis is Ullung Island, Korea (Fig. 1). Located 137 km E of the Korean Peninsula, it is a single oceanic island of 73 km² reaching 984 m tall, and dated radiometrically as 1.8 myo (Kim 1985). It is also quite uniform ecologically (Kim 1988). The level of anagenesis among the 36 endemic species of the island is at least 88% (Stuessy et al. 2006). Noteworthy is that nearly all of the endemic species are the only endemics in their respective genera. The only exceptions are in Acer (Aceraceae), Carex (Cyperaceae), and Viola (Violaceae), with two species each. It is known, however, that in the case of Acer, the two species have each evolved anagenetically from different continental progenitors (Pfosser et al. 2002a). Anagenetic speciation in Ullung Island, therefore, is the dominant form of speciation in the endemic vascular flora, and it is the highest level of anagenesis known in any oceanic island (Table 1).

What is needed at this time is a summary of the results of studies on speciation in Ullung Island, especially at the genetic level. This is an important island, because it represents a case of speciation so very different from the dramatic adaptive radiations in other archipelagos. The purposes of this paper, therefore, are to: (1) survey the genetic patterns within and among populations of endemic plant species on Ullung island; (2) summarize these patterns for an evaluation of the genetic consequences of anagenesis that have taken place; and (3) compare these anagenetic trends with those seen in other oceanic archipelagos.

Population genetics of endemic plant species on Ullung Island

A number of investigations on genetic diversity in endemic species of Ullung Island have been done previously using isozymes (Table 2). Park et al. (2010) investigated genetic variation using eight enzymes within Scrophularia takesimensis, which is an endangered endemic species. High genetic diversity between populations was found. 79 individuals were examined in four populations, which are the only known populations. All four populations gave 11.1% polymorphic loci, an average of 1.18 alleles per locus, mean observed heterozygosity in each of the populations (0.021, 0.000, 0.006, 0.032; overall mean, 0.015), and mean expected heterozygosity (0.020, 0.063,
Table 2. Genetic diversity based on isozymes in endemic species of Ullung Island (bold) plus continental relatives

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of populations sampled</th>
<th>Number of individuals analyzed</th>
<th>( P_R )</th>
<th>( H_E )</th>
<th>( H_O )</th>
<th>( A )</th>
<th>( A_E )</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campanula takesimana</td>
<td>6</td>
<td>300</td>
<td>7.14</td>
<td>0.021</td>
<td>0.014</td>
<td>1.07</td>
<td>1.03</td>
<td>Chung et al. 1998</td>
</tr>
<tr>
<td>C. punctata (Japan, mainland)</td>
<td>7</td>
<td>ca. 325</td>
<td>72.2</td>
<td>0.117</td>
<td>2.39</td>
<td></td>
<td></td>
<td>Inoue and Kawahara 1990</td>
</tr>
<tr>
<td>C. punctata (Japan, Izu Islands)</td>
<td>10</td>
<td>ca. 235</td>
<td>61.1</td>
<td>0.044</td>
<td>1.89</td>
<td></td>
<td></td>
<td>Inoue and Kawahara 1990</td>
</tr>
<tr>
<td>Corydalis filistipes</td>
<td>4</td>
<td>120</td>
<td>61.2</td>
<td>0.167</td>
<td>0.201</td>
<td>1.73</td>
<td>1.20</td>
<td>Seok et al. 2006</td>
</tr>
<tr>
<td>Fagus multinervis</td>
<td>6</td>
<td>300</td>
<td>57.14</td>
<td>0.253</td>
<td>0.211</td>
<td>1.93</td>
<td>1.57</td>
<td>Chung et al. 1998</td>
</tr>
<tr>
<td>F. multinervis</td>
<td>5</td>
<td>152</td>
<td>85.44</td>
<td>0.373</td>
<td>0.383</td>
<td>2.13</td>
<td>1.78</td>
<td>Ohkawa et al. 2006</td>
</tr>
<tr>
<td>F. japonica (Japan)</td>
<td>2</td>
<td>70</td>
<td>0.444</td>
<td>0.456</td>
<td>2.88</td>
<td>1.89</td>
<td></td>
<td>Ohkawa et al. 2006</td>
</tr>
<tr>
<td>Hepatica maxima</td>
<td>6</td>
<td>300</td>
<td>8.70</td>
<td>0.032</td>
<td>0.021</td>
<td>1.08</td>
<td>1.05</td>
<td>Chung et al. 1998</td>
</tr>
<tr>
<td>H. maxima</td>
<td>1</td>
<td>52</td>
<td>50.0</td>
<td>0.058</td>
<td>1.56</td>
<td></td>
<td></td>
<td>Woo et al. 2002</td>
</tr>
<tr>
<td>H. asiatica (Korea)</td>
<td>4</td>
<td>79</td>
<td>79.0</td>
<td>2.76</td>
<td></td>
<td></td>
<td></td>
<td>Chung et al. 1998</td>
</tr>
<tr>
<td>H. asiatica (Korea)</td>
<td>10</td>
<td>337</td>
<td>94.12</td>
<td>0.276</td>
<td>4.06</td>
<td></td>
<td></td>
<td>Kim and Lee 1994</td>
</tr>
<tr>
<td>H. insularis (Korea)</td>
<td>10</td>
<td>508</td>
<td>82.4</td>
<td>0.255</td>
<td>3.35</td>
<td></td>
<td></td>
<td>Kim and Lee 1994</td>
</tr>
<tr>
<td>Scrophularia takesimensis</td>
<td>4</td>
<td>79</td>
<td>11.1</td>
<td>0.036</td>
<td>0.015</td>
<td>1.18</td>
<td></td>
<td>Park et al. 2010</td>
</tr>
</tbody>
</table>

\( P_R \). Per cent polymorphic loci. \( H_E \). Expected proportion of heterozygotes. \( H_O \). observed proportion of heterozygotes. \( A \). Mean number of alleles per locus. \( A_E \). Mean effective alleles per locus.

0.033, 0.028; overall mean, 0.036). The mean \( F_{ST} \) value of the nine loci was 0.158. Nei’s genetic identity (Nei 1978) among the populations ranged from 0.980 to 0.999.

Seok et al. (2006) examined genetic variation in four populations (“subpopulations”) of the outcrossing endemic Corydalis filistipes using seven enzyme systems. The results gave 61.2% polymorphic loci, 0.167 expected proportion of heterozygotes, 0.201 observed proportion of heterozygotes, 1.73 mean number of alleles per locus, and 1.20 mean effective alleles per locus. In addition, the analysis of fixation indices gave an excess of heterozygotes (mean \( F_{IS} \), -0.1889; \( F_{IT} \), -0.1226), and approximately only 5.6% of the variation was distributed among the populations (\( F_{ST} \), 0.0557). Nei’s genetic identity among the four populations ranged from 0.967 to 1.000, emphasizing the lack of geographic partitioning of the genetic variation.

Chung et al. (1998) analyzed six flowering plant species on Ullung Island, of which three are endemic and of interest for this review: Campanula takesimana, Hepatica maxima, and Fagus multinervis. Six populations of each species were sampled with 50 plants from each population, giving a rather large sample in each species of 300 individuals. Thirteen enzyme systems were successfully scored. In Campanula takesimana, an herbaceous species, the data show 7.14% polymorphic loci, 0.021 mean expected heterozygosity, 0.014 mean observed heterozygosity, 1.07 mean number of alleles per locus, and 1.03 mean effective number of alleles per locus. This is a relatively low level of genetic variation among the endemic species. Analyses of the close continental relative, Campanula punctata, from seven populations from mainland Japan, and 10 from the Izu Islands (Inoue and Kawahara 1990), revealed percent polymorphic loci of 72.2% and 61.1%, respectively, which is much higher than in C. takesimana of Ullung Island. The mean expected heterozygosity is 0.123, which is considerably higher than in C. takesimana. In an earlier study, Inoue and Kawahara (1990) examined 10 Japanese populations of C. punctata from Izu Islands, and these gave a much reduced 0.088 expected proportion of heterozygotes.

In Hepatica maxima, Chung et al. (1998) found that the percentage of polymorphic loci was 8.70, mean expected heterozygosity 0.032,
observed mean heterozygosity 0.021, mean number of alleles per locus 1.08, and mean effective number of alleles per locus 1.05. Genetic variation in this herbaceous species, therefore, is somewhat low. In comparison, Woo et al. (2002) examined a population of *Hepatica maxima* in Ullung Island, sampling 52 plants, and their data yielded 50% polymorphic loci, 0.058 expected proportion of heterozygotes, and 1.56 mean number of alleles per locus. Total gene diversity was low. Kim and Lee (1994) also sampled and analyzed 10 populations with 10 enzyme systems of the Korean peninsular *H. asiatica*, which gave 94% polymorphic loci, a 4.06 mean number of alleles per locus, and a 0.276 expected proportion of heterozygotes in this species. They also investigated the closely related *H. insularis*, which is confined primarily to the southern end of the Korean peninsula. The values obtained were 82.4% polymorphic loci, a 3.35 mean number of alleles per locus, and a 0.255 expected proportion of heterozygotes. In Woo et al. (2002) the same data were reanalyzed and provided a range of genetic identities among populations within *H. asiatica* of 0.9313–0.9637 (mean 0.9480) and within *H. insularis* 0.6500–0.9838 (mean 0.7794). Between the two species the values averaged 0.7514, which partially supports recognition of these populational systems as distinct species. The relationship between them, however, is not entirely clear (Pfosser et al. 2011), and this might be due to hybridization after secondary contact during the Pleistocene. Molecular phylogenetic studies have indicated that *H. asiatica* is the closest relative of the endemic *H. maxima* (Pfosser et al. 2011).

Chung et al. (1998) also investigated isozymic variation in *Fagus multinervis*, a large dominant forest tree in the island, which resulted in 57.14% polymorphic loci, 0.253 mean expected heterozygosity, 0.211 mean observed heterozygosity, 1.93 mean number of alleles per locus, and 1.57 mean effective number of alleles per locus. The level of genetic variation in this endemic species, therefore, is quite high. Ohkawa et al. (2006) also investigated genetic variation in *Fagus multinervis* using 11 allozymes within 152 plants in five populations. The results gave 85.44% mean polymorphic loci, 0.373 expected proportion of heterozygotes, 0.383 observed proportion of heterozygotes, 2.13 mean number of alleles per locus, and 1.78 mean effective alleles per locus. Wright’s (1922) inbreeding coefficient (mean) yielded $F_{IS}$, $−0.013$, which suggests a high level of gene flow among the populations. Furthermore, $G_{ST}$, the proportion of genetic diversity residing among populations, was calculated as 0.036. In a related study and for comparison with the island endemic, Ohkawa et al. (2006) also examined two populations of *F. japonica*, one of the closest relatives from Japan (Oh 2015), and found 0.444 to be the expected proportion of heterozygotes, 0.456 the observed proportion of heterozygotes, 2.88 the mean number of alleles per locus, and 1.89 the mean effective alleles per locus. $G_{ST}$ was estimated to be 0.083, showing a somewhat higher subdivision of genetic variation between the two populations studied. The level of genetic variation in the endemic species, therefore, is slightly lower than in the Japanese relative. At the nucleotide level in *F. multinervis*, however, Oh (2015) recently examined *psbA-trnH* chloroplast sequences and found very low haplotype diversity.

These isozyme studies are useful in providing a general view of patterns of genetic variation within these species, but the results are not precise enough to allow careful comparisons among species nor between types of speciation. Molecular markers that reveal much more of the genotype, such as RAPDs, AFLPs and SSRs, are needed for these evaluations. As an example of this problem, we mention the isozyme studies and SSR investigations done on the endemic *Lactoris fernandeziana* in the Juan Fernández Archipelago. With isozymes (Crawford et al. 1994) almost no genetic variation was detected within and among populations on Robinson
crusoe island. with rapds (brauner et al. 1992) and isssrs (crawford et al. 2001), however, considerable variation was detected. hence, it is nearly impossible to compare directly the results of aflps, ssrs and isozymes in this archipelago or in any island region.

rapd population markers were used by ku et al. (2004) to analyze the only two known populations of the ullung endemic bupleurum latissimum nakai. this is a rare outcrossing, entomophilous perennial herb. one population contained 45 individuals that gave 0.146 nei’s gene diversity and 62.5% polymorphic bands. the second population with 61 individuals showed 0.151 nei’s gene diversity and 56.0% polymorphic bands. the amova for the species within populations was 65.9% and between populations 34.1%. the overall conclusion, therefore, is that this species harbors a relatively high level of genetic variation, and that most of the variation is found within the populations rather than between them.

aflp investigations have been completed on two endemic species of ullung island: dystaenia takesimana (pfosser et al. 2006) and hepatica maxima (pfosser et al. 2002b, 2011, unpubl.) (table 3). hepatica maxima has been examined in detail with aflps, but these data are not yet fully published. in an abstract (pfosser et al. 2002b), a high number of private alleles was reported in comparison to h. asiatica on the korean peninsula, and the genetic variation in the island was not partitioned geographically.

results from pfosser et al. (2006) with dystaenia reveal that in d. takesimana the percentage of polymorphic fragments varies from 93.33 to 98.39 (mean 96.13), and in d. ibukiensis this value varies from 84.62 to 88.98 (mean 86.73). the shannon diversity index gave 31.91 to 33.74 (mean 33.13) in d. takesimana and 27.04 to 30.48 (mean 29.13) in d. ibukiensis. the number of polymorphic fragments, the percent of polymorphic fragments, the shannon diversity index, and the average gene diversity over loci are all higher in dystaenia takesimana in comparison to the japanese d. ibukiensis. it is of interest that morphological studies conducted by sun et al. (1997) have shown that, in contrast to the genetic results, leaves are less variable in d. takesimana than in d. ibukiensis. this anomaly might be explained by the absence of selection for diverse habitats in the relatively uniform island environment in contrast to the broader ecological differences within mainland japan.

as for microsatellites (ssrs), studies have been completed on acer takesimensis

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Table 3. Genetic diversity based on AFLPs and SSRs in anagenetically derived species of Ullung Island (bold) and close continental relatives

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of populations sampled</th>
<th>Number of individuals analyzed</th>
<th>AFLPs</th>
<th>SSRs</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>PR</td>
<td>SDI</td>
<td>HE</td>
</tr>
<tr>
<td>Acer okamotoanum</td>
<td>7</td>
<td>145</td>
<td>0.72</td>
<td>6.60</td>
<td>0.18</td>
</tr>
<tr>
<td>A. mono (Korea and Japan)</td>
<td>7</td>
<td>134</td>
<td>0.80</td>
<td>8.37</td>
<td>0.27</td>
</tr>
<tr>
<td>A. takesimense</td>
<td>7</td>
<td>130</td>
<td>0.53</td>
<td>0.38</td>
<td>3.82</td>
</tr>
<tr>
<td>A. pseudosieboldianum (Japan)</td>
<td>7</td>
<td>133</td>
<td>0.61</td>
<td>0.40</td>
<td>4.60</td>
</tr>
<tr>
<td>Dystaenia takesimana</td>
<td>6</td>
<td>62</td>
<td>96.13</td>
<td>33.13</td>
<td></td>
</tr>
<tr>
<td>D. ibukiensis (Japan)</td>
<td>6</td>
<td>64</td>
<td>86.73</td>
<td>29.13</td>
<td></td>
</tr>
</tbody>
</table>

PR. Percent polymorphic fragments. SDI. Mean Shannon Diversity Index. HE. Expected proportion of heterozygotes. HO. Observed proportion of heterozygotes. AR. Allelic richness. FIS. Inbreeding coefficient. TA. Total number of alleles.
(Takayama et al. 2012a), and Acer okamotoanum (Takayama et al. 2012b) (Table 3). In these cases, comparisons have been made with continental close relatives, i.e., A. pseudosieboldianum, and A. mono, respectively. In A. okamotoanum vs. A. mono the genetic diversity statistics reveal, respectively: $H_E$ as 0.72 vs. 0.80; $A_R$, 6.80 vs. 8.37; $F_{IS}$, 0.18 vs. 0.27; and $T_A$, 57 vs. 72. In A. takesimense vs. A. pseudosieboldianum, the genetic diversity statistics reveal, respectively: $H_E$ as 0.53 vs. 0.61; $H_O$, 0.38 vs. 0.40; $A_R$, 3.82 vs. 4.60; $F_{IS}$, 0.28 vs. 0.33; and $T_A$, 35.3 vs. 42.3. All these data indicate that the mainland Japanese and Korean related species harbor slightly more genetic variation than the Ullung Island endemic. AMOVA results show that the genetic variation is apportioned mostly within populations in A. okamotoanum (96%) in contrast to A. mono with 89.5%. In the other species pair, the island A. takesimense also contains 96.2% of genetic variation within populations, and the mainland relative 93.5%. These data indicate considerable gene flow among individuals on the island that prevents geographic structuring. The mainland species in both cases, especially with A. pseudosieboldianum, have more variation accounted for among populations.

**Anagenetic speciation in the endemic flora of Ullung Island**

To properly consider speciation in Ullung Island, it is necessary to first deal briefly with taxonomic considerations. A number of recent floristic and revisionary studies have attempted to refine estimates of endemic vascular plants in Ullung Island (Jung et al. 2013, Yoon et al. 2013, Chang and Gil 2014, Sun et al. 2014, Yang et al. 2015). These more recent contributions have documented 30–33 endemic taxa, fewer than the 37 species listed previously (Sun and Stuessy 1998). The reason for different estimates is due, in part, to different concepts of species. Because Ullung is a young volcanic island, being only 1.8 million years old, only a short period of time has existed for morphological divergence to occur. Regarding Hepatica maxima, despite the conspicuous morphological difference between the endemic species and the Korean peninsula H. asiatica, the former has been treated by some workers as either a form or variety of the latter (Park 1974, Lee 1976). Some workers have also transferred the species to Anemone (e.g., Sun et al. 2014). In Acer, the endemic A. takesimense has been regarded as a good species (Park et al. 1993, Pfosser et al. 2002a, Takayama et al. 2012a) or as a subspecies of A. pseudosieboldianum (de Jong 1994, Yang et al. 2015), to which it is clearly related evolutionarily (Pfosser et al. 2002a, Takayama et al. 2012a). Likewise, the endemic A. okamotoanum has been relegated by Ohashi (2002) to a subspecies of A. pictum Thunb., although previous molecular studies by Pfosser et al. (2002a) suggested that it belonged most closely to A. mono. Denk (2003), based on morphological analyses, regarded Fagus multinervis as a synonym of F. engleriana, but the isozyme data presented by Ohkawa et al. (2006) would not support that action.

Evaluation of patterns of genetic variation for purposes of understanding genetic consequences of anagenetic and cladogenetic speciation within endemic island species is not a simple task, and many factors may be responsible (Stuessy et al. 2014). Population size is obviously significant, with larger populations containing more variation than smaller ones. The type of breeding system is also important, as inbreeders will nearly always harbor lower levels of genetic variation than outcrossers (Hamrick et al. 1991). The age of the species can also affect the degree of genetic diversity, especially in endemic island species. From an initial founder effect upon arrival and establishment in an island, genetic variation accumulates and is channeled depending upon whether the ecological setting provides avenues for cladogenesis or only anagenesis. The type of speciation, therefore, is particularly influential in the pattern of genetic diversity that results. Added to these natural
factors is the human disturbance on an island, which can have a major impact on reduction of habitat, loss of populations or population size, and loss of genetic diversity. For comparisons among species, therefore, care must be exercised in drawing conclusions. This is particularly the case when comparing results between species on different archipelagos, which themselves may have had very different geological and ecological ontogenies (Stuessy 2007).

The isozyme data available for endemic species of Ullung Island (Table 2) clearly show that the island endemics harbor less genetic variation than their continental close relatives. Particularly conspicuous in this regard is *Hepatica maxima*. Chung et al. (1998), for example, obtained a value of 8.70 for percent polymorphic loci and 79 for *H. asiatica*. Woo et al. (2002) found values of 50.0 for *H. maxima*, a much higher value, but also a very high 94.12 for *H. asiatica*. The conclusions in these cases emphasized the genetic bottleneck resulting from the initial founder effect, which has often been invoked to explain limited genetic variation in endemic species of oceanic islands. As pointed out by Stuessy et al. (2012), however, as time continues after initial colonization, and speciation ensues (by whichever means), it is unlikely that the restricted genetic signature from the original founding event would still be evident.

The data from the molecular markers RAPDs, AFLPs and SSRs from species in Ullung Island, however, reveal a different picture than that suggested by isozymes. Several trends can be seen (Table 3). First, the Ullung Island endemics all show clear morphological and genetic differences from their continental close relatives. This bears on the taxonomic points mentioned earlier, and the genetic data provide support for specific recognition in these three cases. Anagenetic speciation is a slow process in which the developing population simply enlarges and accumulates genetic variation over time, and in the process some morphological features change that eventually allow designation of distinct species.

Second, the overall levels of genetic variation can vary from species to species. *Dystaenia takesimensis* has relatively high levels, in fact, higher than in its close relative, *D. ibukiensis*. In the two species of *Acer*, however, the levels of genetic diversity are lower than their close continental relatives. In no cases, however, are the levels of genetic diversity in the island endemics depauperate. When the original colonists to the island arrived some 1.8 million or less years ago, surely there was an initial founder effect. Over time, however, this strong reduction from the continental gene pools was no longer evident as the growing population accumulated genetic variation.

Third, among the subpopulations on the island, no geographic patterning to the genetic variation is seen. That is, the profile is always a mosaic with the genetic diversity varying in no particular geographic pattern. This would be in sharp contrast to groups that have adaptively radiated, in which each species is contained in a small portion of the island in a particular ecological zone, and each harboring a distinct genetic pattern. Adaptive radiation, however, is unknown in Ullung Island. Genetic partitioning does not occur within species diverging anagenetically because the population simply keeps growing and accumulating genetic diversity over time. Gene flow continues among the subpopulations and promotes homogeneity. Mutation, recombination and drift are important in establishing the levels of variation seen. Likewise, the morphology is also uniform over the landscape, which would be expected in an environment lacking distinct ecological zones where radiations could take place.

**Comparison of anagenetic speciation in Ullung Island with other island system**

Despite the numerous studies that have been completed on adaptive radiation in endemic plant taxa, only a few have examined the
genetic consequences resulting from anagenesis. Aside from Ullung Island, the archipelago in which similar studies have been carried out is the Juan Fernández Archipelago. This island system consists of two major islands: Robinson Crusoe Island and Alejandro Selkirk Island. They are approximately the same size (50 km²) but the geological ages are different, with ca. 4 myo for the former and 1–2 myo for the latter (Stuessy et al. 1984). Studies on the genetic consequences of anagenesis in this archipelago have been completed for: *Drimys confertifolia* (Winteraceae; López-Sepúlveda et al. 2014), *Myrceugenia fernandeziana* and *M. schulzei* (Myrtaceae; López-Sepúlveda et al. 2013), *Rhaphithamnus venustus* (Verbenaceae; López-Sepúlveda et al. in press), and *Robinsonia masafuerae* (Takayama et al. 2015a). Comparison between anagenetic and cladogenetic consequences in Juan Fernández have also been summarized recently (Takayama et al. 2015b). The advantage of these studies is that they have been done using the same methods in the same island system, and therefore, it is easier to compare and contrast results. Likewise, these methods are basically the same as those used for the AFLP and SSR data from Ullung Island. Hence, comparisons between species in these two island systems are more meaningful than with attempts to compare genetic variation examined by other methods in species of other archipelagos with very different geological histories (e.g., Hawaiian Islands).

As with Ullung Island, isozyme studies have also been completed on quite a number of the endemic species of the Juan Fernández Archipelago (Crawford et al. 2001). In context of anagenetic speciation, however, only three of the anagenetically evolved species have been investigated: *Dysopsis hirsuta*; *Myrceugenia fernandeziana*; and *Rhaphithamnus venustus*. *Lactoris fernandezianus* is also the only species of its genus in the islands, and therefore rightly would fall into the category of an anagenetically originated species. This is a very old relict taxon, however, that has a very different evolutionary history (Stuessy et al. 1998) and hence is inappropriate for comparisons of genetic consequences during speciation.

Results from the isozyme analyses in the three anagenetically evolved species in the Juan Fernández Archipelago are of interest (Table 4), but they cannot be related directly to those from AFLPs and SSRs because of the greater sampling of the genome of the latter. The species level diversity (*H*<sub>ES</sub>) within *Dysopsis hirsuta*, *Myrceugenia fernandeziana*, and *Rhaphithamnus venustus* are 0.039, 0.091, and 0.028, respectively. These are relatively low values, typical of species in Pacific Islands (de Joode and Wendel 1992, Frankham 1997). Mean population diversity (*H*<sub>EP</sub>) in these species is: 0.022, 0.056, and 0.022; the proportion of diversity residing among populations (*G*<sub>ST</sub>) is: 0.299, 0.232, 0.224; and the proportion of polymorphic loci (*P*<sub>R</sub>) is: 0.30, 0.67, and 0.17. These results point to very low genetic variation within these species in general. *Dysopsis hirsuta* is a perennial, wind-pollinated herb (Bernardello et al. 2001) occurring in isolated populations (patches) in small clearings of the *Myrceugenia* forests. *Rhaphithamnus venustus* is a gynodioecious (Sun et al. 1996), thin tree to 5 m tall, hummingbird-pollinated (Anderson et al. 2001), and occurring in small populations. *Myrceugenia fernandeziana* is a large tree, often growing in large populations, and being the dominant forest tree on Robinson Crusoe Island. It is also apparently wind-pollinated, based on lack of insects seen in pollination observations (Skottsberg 1928). This species shows the highest level of genetic diversity among the three taxa.

Use of AFLP and SSR molecular markers in the Juan Fernández Archipelago also allows greater precision in examining levels of intrapopulational variation within the anagenetically derived species. Table 5 presents genetic diversity statistics for five anagenetic species.
Table 4. Genetic diversity in anagenetically derived species of the Juan Fernández Archipelago based on allozymes. From Crawford et al. (2001)

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of populations studied</th>
<th>$P_p$</th>
<th>$H_{ES}$</th>
<th>$H_{EP}$</th>
<th>$G_{ST}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Dysopsis hirsuta</em></td>
<td>21</td>
<td>0.30</td>
<td>0.039</td>
<td>0.022</td>
<td>0.299</td>
</tr>
<tr>
<td><em>Myrceugenia fernandeziana</em></td>
<td>19</td>
<td>0.67</td>
<td>0.091</td>
<td>0.056</td>
<td>0.232</td>
</tr>
<tr>
<td><em>Rhaphithamnus venustus</em></td>
<td>14</td>
<td>0.17</td>
<td>0.028</td>
<td>0.022</td>
<td>0.224</td>
</tr>
</tbody>
</table>


Looking at all the anagenetic species in the Juan Fernández Archipelago (Table 5), several general trends can be seen. First, anagenetically derived endemic species have similar/slightly higher average gene diversity over loci (AGDOL) than that of related continental species. Second, the coefficient of inbreeding shows that the lowest values occur in *Robinsonia masafuerae* and *Rhaphithamnus venustus*, which are the two species with atypical breeding systems, dioecious and gynodioecious, respectively. The highest values are in the two species of *Myrceugenia* and *Drimys confertifolia*, all large trees occurring in large populations.

Third, because data exist for endemic species on both of the Juan Fernández Islands, one older than the other, it allows this time dimension to be examined in the data. Differences between the species do occur, and this is particularly seen in *D. confertifolia* which has greater genetic diversity on the older island in six parameters (SDI, AGDOL, $H_E$, $H_O$, $A_R$, $N_A$, Table 5). These data suggest that over time, populations on the older island during the past four million years have been accumulating genetic diversity. These levels during the 1–2 million years on Alejandro Selkirk Island have not yet risen to the levels on the older island.

Determination of close continental relatives of the endemic island species, putative progenitors (or direct descendents of them), allows comparisons to be made in genetic diversity statistics (Table 5). In *Myrceugenia* and *Drimys*, the anagenetically evolved island endemic species is considerably more genetically diverse than the continental progenitor. In the case of *Robinsonia, R. masafuerae* has evolved from *R. evenia*, a cladogenetically evolved island endemic in the older island. This represents an anagenetic speciation event that has taken place during the past 1–2 million years (the maximum age of Alejandro Selkirk Island). Here the two species are very similar in genetic diversity. This is an interesting case because *R. evenia* does not harbor broad diversity, having been derived from splitting events over the past four million years (Takayama et al. 2015a). From out of this cladogenetic complex, therefore, has dispersed propagules to the younger island, when formed from the hotspot in the ocean, and *R. masafuerae* has accumulated genetic variation now nearly equaling its progenitor species. *Rhaphithamnus venustus* is another interesting situation. The earlier studies on isozymes showed that the endemic species had much lower genetic variation than the continental *R. spinosus* (Crawford et al. 1993b). The AFLP and SSR data (Table 5) also suggest that the island species harbors less genetic variation than the mainland relative. Some of the microsatellite statistics ($A_R$, $F_{IS}$, and $N_A$), however, show the reverse trend.

**Conclusions**

The data available for anagenetically derived species in Ullung Island, plus those species now also analyzed for the Juan Fernández Archipelago, allow a summary to be made.
regarding genetic diversity in species with this type of mode of speciation. The advantage of investigating in Ullung Island is that nearly all, if not all, of the endemic species have evolved anagenetically. The situation in the Juan Fernández Archipelago is useful because it permits a direct comparison between the two modes of speciation in the same island system with the same population genetics techniques. Furthermore, there are two principal islands of differing geological ages.

The first general point from these studies is that the level of genetic variation in anagenetically evolved species is normally quite high. Despite that the initial founder effect must have greatly reduced the incoming genetic variation in comparison to the range from the progenitor population, over millions of years, the level of genetic diversity has increased. Gene flow is maintained over the landscape among the subpopulations, such that with mutation and recombination, a broader population forms containing considerable genetic variation. There is no partitioning of this growing gene pool into discrete and isolated populations, such as is the case with cladogenesis.

The second important point is that owing to gene flow broadly connecting the subpopulations on the island, no distinct genetic partitioning can be seen. Rather, a mosaic of genetic variation develops. There is no north-south or east-west patterning seen in all of the species examined.

The third point to emphasize is that the amount of genetic variation that may be documented in an anagenetic species should be directly related to its age. If genetic variation accumulates over time, the degree of diversity should be correlated with the size of the growing population, which probably relates somewhat to the size of the island, and also the geological age of the island. One would expect more genetic variation in species on an older island rather than on a younger island. The best evidence of this is seen in a comparison of *Drimys confertifolia* and *Rhaphithamnus venustus*, which occur on both islands of the Juan Fernández Archipelago.
In both cases much more genetic variation is found in Robinson Crusoe Island, which is twice the geological age of Alejandro Selkirk Island. A recently evolved species should have less genetic variation than an older one. Considerable time has to pass, obviously, before enough genetic and morphological distinctness appears such that the island population is considered a new species. Prior to that point, the island taxon would be still conspecific with its progenitor.

It is also important to remember, however, that a counter-trend may be taking place over geological time. Reduction of island size and elevation due to plate subsidence and erosion will have a marked impact on lowering habitat diversity (Stuessy et al. 2005). An older island, therefore, may have more restricted original vegetation zones in comparison to those on a younger island, such as can be seen clearly in the Juan Fernández Archipelago (Greimler et al. 2002, 2013). This change over time may result in fewer or smaller populations and overall loss of genetic diversity.

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韓国鬱陵島固有植物の遺伝的多様性と種分化

系統進化のパターンに注目すると、海洋島での種分化には2つの様式が見られる。一つは適応放散的種分化に代表されるcladogenesis（分岐を伴う種分化）で、島に侵入した祖先が複数の種に分化している様式である。もう一つはanagenesis（分岐を伴わない種分化）で、島に侵入した祖先系統が分岐せずに時間と共に変化し、母種とは異なる種に分化している様式である。anagenesisに起源する固有種の遺伝的特徴を調べた研究は極めて少なく、cladogenesisに起源する固有種の遺伝的特徴と比較することで、海洋島における種分化過程の全貌が明らかになっていくことが期待されている。著者ちは固有種のほとんどが分岐を伴わない種分化を遂げたと推測されている韓国の鬱陵島の植物に着目し、AFLPやマイクロサテライトマーカーを用いて、その遺伝的特徴を明らかにした。鬱陵島固有種は大陸産の近縁種と同程度の遺伝的多様性を保持しており、島内の集団分化もほとんど見られなかった。この結果は、anagenesisに起源する固有種が局所適応によらず、大きな集団を保ちながら突然変異を蓄積し、種分化したことを示唆している。

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