Global patterns of genetic variation in plant species along vertical and horizontal gradients on mountains

Takafumi Ohsawa* and Yuji Ide

ABSTRACT

Aim To understand global patterns of genetic variation in plant species on mountains and to consider the significance of mountains for the genetic structure and evolution of plant species.

Location Global.

Methods We review published studies.

Results Genetic diversity within populations can vary along altitudinal gradients in one of four patterns. Eleven of 42 cited studies (26% of the total) found that populations at intermediate altitudes have greater diversity than populations at lower and higher altitudes. This is because the geographically central populations are under optimal environmental conditions, whereas the peripheral populations are in sub-optimal situations. The second pattern, indicating that higher populations have less diversity than lower populations, was found in eight studies (19%). The third pattern, indicating that lower populations have lower diversity than higher populations, was found in 10 studies (24%). In 12 studies (29%), the intrapopulation genetic variation was found to be unaffected by altitude. Evidence of altitudinal differentiation was found in more than half of these studies, based on measurements of a range of variables including genome size, number of chromosomes or a range of loci using molecular markers. Furthermore, great variation has been found in phenotypes among populations at different altitudes in situ and in common garden experiments, even in cases where there was no associated variation in molecular composition. Mountains can be genetic barriers for species that are distributed at low elevations, but they can also provide pathways for species that occupy high-elevation habitats. [Correction added after publication 9 October 2007: ‘less diversity’ changed to ‘greater diversity’ in the second sentence of the Results section of the Abstract].

Main conclusions Genetic diversity within populations can vary along altitudinal gradients as a result of several factors. The results highlight the importance of phenotypic examinations in detecting altitudinal differences. The influence of mountain ridges on genetic differentiation varies depending, inter alia, on the elevation at which the species occurs. Based on these findings, zoning by altitudes or ridges would be helpful for the conservation of tree populations with the onset of global warming.

Keywords Altitudinal gradient, biogeography, genetic variation, mountains, phenotype, plant species.

INTRODUCTION

Variation within a number of plant species along altitudinal gradients has been studied for several purposes (e.g. Isik & Kara, 1997; Senjo et al., 1999; Sáenz-Romero & Tapia-Olivares, 2003; Jump et al., 2006; Sáenz-Romero et al., 2006; Truong et al., 2007). One of the main objectives with regard to tree species has been to identify suitable populations that can be used in tree breeding programmes to provide high-quality wood quickly, and phenotypic examination in common garden experiments is one
classical method (Isik & Kara, 1997; Sáenz-Romero et al., 2006).
In recent years, however, many studies of genetic variation along altitudinal ranges have also been performed with the aid of neutral molecular markers (Senjo et al., 1999; Jump et al., 2006; Truong et al., 2007). The information obtained in such studies can be very important in several respects. Notably, it can help to assess the distribution, genetic structure and evolution of mountain populations (Alden & Loostra, 1987; Jump et al., 2006; Truong et al., 2007) and provide helpful indicators of appropriate conservation strategies for them (Wen & Hsiao, 2001; Sáenz-Romero & Tapia-Olivares, 2003).

Nonetheless, the factors affecting variation within species are so diverse that the results of the published studies are highly varied (e.g. Ohsawa et al., in press) and complex (e.g. Williams & Arnold, 2001). The major patterns of variation within and among populations of specific species on mountains have not been clearly described to date, and thus it has been difficult to discuss the results of specific case studies in relation to general patterns. In this paper we review published studies and collate them in order to assess and describe general, global patterns of genetic variation on mountains and to promote related research in the future. The data examined in this review also allow us to consider the significance of mountains for the genetic structure and evolution of plant species. In this context, the following questions are addressed: (1) does intrapopulation genetic variation change along altitudinal gradients and, if so, why; (2) does interpopulation genetic variation exist at different altitudes and, if so, why; and (3) does interpopulation phenotypic variation exist at different altitudes in common garden experiments and, if so, why?

These three questions focus on the genetic changes in plant populations on mountains along vertical axes, but genetic changes can also occur along horizontal axes. For instance, ridges may provide geographical barriers to gene flow between populations on their opposite sides, so genetic differentiation may occur across ridges (Taberlet et al., 1998). Hence, the following question was also addressed: (4) what genetic changes occur across mountains along horizontal axes?

To describe global patterns of genetic variation, we review published studies on both phenotypic and genetic variations. In general, phenotypic variation can be divided into environmental and genetic variation (Via et al., 1995; Pigliucci, 1996). The phenotypic differences observed in situ reflect both types of variation, while the variation observed in common garden experiments reflects only genetic variation. In this paper we do not cite the studies on phenotypic variations observed in situ to preclude the effect of environmental variation. The major forces determining genetic variation are mutation, genetic drift (including bottlenecks), gene flow and natural selection, though other minor forces are also known (cf. Frankham et al., 2004; Lowe et al., 2004). Non-neutral loci are under natural selection, and thus phenotypic variations governed by them are also affected by selection. However, neutral loci are generally unaffected by natural selection, though the degree to which neutral markers are valuable surrogates for traits under natural selection is unclear.

CHANGES IN INTRAPOPULATION GENETIC VARIATION WITH ALTITUDE

To consider the first and second questions posed in the Introduction, we reviewed a number of published case studies. Here, we mainly considered studies of tree species using neutral molecular markers performed at regional scales (of the order of 1–10 km) (Table 1). Studies at larger scales (> 102 km) were not considered, because large horizontal distances seem to affect genetic variation more than limited vertical distances. Some of the studies listed in Table 1 did not specifically focus on relationships between altitude and genetic parameters, but we used them if information concerning such relationships could be extracted from them. In addition, similar studies of herbaceous species are listed (Table 2). In almost all of these case studies, one of four patterns of intrapopulation genetic variation, generally represented by expected heterozygosity, can be discerned: ‘L < M > H’, ‘L > M > H’, ‘L < M < H’ or ‘L = M = H’, where L, M and H represent low, middle and high altitudes, respectively (Fig. 1), and =, < and > represent roughly equal, increasing and decreasing genetic diversity with increasing altitude, respectively.

Eleven of 42 cited studies (26% of the total) showed that ‘L < M > H’, indicating that the lower and higher populations have less diversity than the populations at intermediate elevations. Isik & Kara (1997) analysed isozymes in four populations of Pinus brutia at elevations ranging from 350 m to 1000 m, and found heterozygosity levels to be greatest in the populations at the intermediate elevations. Similarly, Sáenz-Romero & Tapia-Olivares (2003) investigated genetic variations in isoenzymes among five populations of Pinus oocarpa along an altitudinal gradient from 1100 m to 1500 m, and showed that the population at 1200 m had the greatest average number of alleles per locus. Theoretically, this tendency can be explained in terms of the position of the populations in relation to the range of the species along altitudinal clines; peripheral populations are also ecologically marginal in many cases (Lesica & Allendorf, 1995). The geographically central populations experience optimal conditions, whereas the peripheral populations are generally under suboptimal conditions. In the peripheral populations, limitations on gene flow, population size and the founder effects all promote genetic drift, thereby reducing genetic variation and increasing the differentiation of populations (Lesica & Allendorf, 1995). As a consequence, relatively large genetic variation can be maintained only in the core populations. The studies reporting this pattern went on to suggest that populations at middle altitudes should be selected for conservation purposes, as a gene resource management unit. Some previous studies have also found better seedling development (germination and growth) in middle-elevation populations than in those at lower and higher elevations (Isik & Kara, 1997).

The second pattern is ‘L > M > H’, which indicates that higher populations have relatively small diversity. This was found in eight studies (19%). Quiroga & Premoli (2006) found reductions in the genetic diversity of Podocarpus parlatorei towards the north and on high mountains. A reduction in genetic variation is predicted during forest movements, as a result of bottlenecks
Table 1 Published case studies on genetic variation in tree species on mountains.

<table>
<thead>
<tr>
<th>Species</th>
<th>Altitudinal range (m)</th>
<th>Population number</th>
<th>Polymorphic marker (number of alleles or polymorphic bands)*</th>
<th>Diversity change†</th>
<th>Differentiation‡</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abies balsamea</td>
<td>854 1464</td>
<td>4</td>
<td>8 isozyme loci (2.4)</td>
<td>L &gt; M = H</td>
<td>–</td>
<td>Neale &amp; Adams (1985)</td>
</tr>
<tr>
<td>Abies lasiocarpa</td>
<td>1350 1800</td>
<td>9</td>
<td>9 isozyme loci (3.0)</td>
<td>L &gt; M &gt; H</td>
<td>Δ</td>
<td>Ettl &amp; Peterson (2001)</td>
</tr>
<tr>
<td>Betula occidentalis</td>
<td>– – 33</td>
<td></td>
<td>19 enzyme loci (4.4)</td>
<td>L &gt; M &gt; H</td>
<td>ϕ</td>
<td>Williams &amp; Arnold (2001)</td>
</tr>
<tr>
<td>Betula pubescens var.</td>
<td>450 1200</td>
<td>3</td>
<td>9 nSSR (26.3)</td>
<td>L = M = H</td>
<td>–</td>
<td>Truong et al. (2007)</td>
</tr>
<tr>
<td>Chamaecyparis obtusa</td>
<td>1000 2010</td>
<td>4</td>
<td>6 isozyme loci (2.0)</td>
<td>L = M = H</td>
<td>–</td>
<td>Iide &amp; Katsuki (1992)</td>
</tr>
<tr>
<td>Cryptomeria japonica</td>
<td>900 2050</td>
<td>4</td>
<td>9 alloverge loci (3.0)</td>
<td>L &lt; M &gt; H</td>
<td>ϕ</td>
<td>Taira et al. (1997)</td>
</tr>
<tr>
<td>Fagus multinervis</td>
<td>500 900</td>
<td>5</td>
<td>11 alloverge loci (2.5)</td>
<td>L &lt; M &lt; H</td>
<td>Δ†</td>
<td>Ohkawa et al. (2006)</td>
</tr>
<tr>
<td>Fagus sylvatica</td>
<td>150 660</td>
<td>6</td>
<td>16 isozyme loci (2.7)</td>
<td>L = M &lt; H</td>
<td>Δ</td>
<td>Sander et al. (2000)</td>
</tr>
<tr>
<td>Fagus sylvatica</td>
<td>992 1640</td>
<td>3</td>
<td>5 AFLP primers</td>
<td>L &lt; M &lt; H</td>
<td>Δ</td>
<td>Jump et al. (2006)</td>
</tr>
<tr>
<td>Nothofagus pumilio</td>
<td>385 1630</td>
<td>14</td>
<td>8 enzyme loci (2.8)</td>
<td>L &gt; M &gt; H</td>
<td>ϕ</td>
<td>Premoli (2003)</td>
</tr>
<tr>
<td>Picea abies</td>
<td>830 1500</td>
<td>10</td>
<td>8 isozyme loci (3.0)</td>
<td>L &lt; M &gt; H</td>
<td>–</td>
<td>Gommery (1992)§</td>
</tr>
<tr>
<td>Picea abies</td>
<td>1140 1780</td>
<td>20</td>
<td>18 enzyme loci (2.5)</td>
<td>L = M = H</td>
<td>–</td>
<td>Müller-Starck (1995)</td>
</tr>
<tr>
<td>Picea abies</td>
<td>960 1520</td>
<td>3</td>
<td>5 nSSR (27.0)</td>
<td>L &lt; M &lt; H</td>
<td>Δ</td>
<td>Maghuly et al. (2006)</td>
</tr>
<tr>
<td>Picea abies</td>
<td>960 1520</td>
<td>3</td>
<td>1 mitochondrial primer (2.0)</td>
<td>L = M &lt; H</td>
<td>–</td>
<td>Maghuly et al. (2006)</td>
</tr>
<tr>
<td>Picea glauca</td>
<td>120 750</td>
<td>4</td>
<td>12 enzyme loci (3.2)</td>
<td>L = M &lt; H</td>
<td>×</td>
<td>Alden &amp; Loopstra (1987)</td>
</tr>
<tr>
<td>Picea mariana</td>
<td>– – 10</td>
<td></td>
<td>13 alloverge loci</td>
<td>L &gt; H</td>
<td>–</td>
<td>O’reilly et al. (1985)</td>
</tr>
<tr>
<td>Pinus brutia</td>
<td>350 1000</td>
<td>4</td>
<td>23 isozyme loci</td>
<td>L &lt; M &gt; H</td>
<td>–</td>
<td>Isik &amp; Kara (1997)</td>
</tr>
<tr>
<td>Pinus brutia</td>
<td>275 1050</td>
<td>9</td>
<td>17 isozyme loci (2.9)</td>
<td>L &lt; M &gt; H</td>
<td>–</td>
<td>Kara et al. (1997)</td>
</tr>
<tr>
<td>Pinus brutia</td>
<td>100 975</td>
<td>19</td>
<td>20 RAPD primers (5.2)</td>
<td>L = M = H</td>
<td>×</td>
<td>Kandemir et al. (2004)</td>
</tr>
<tr>
<td>Pinus flexilis</td>
<td>1650 3350</td>
<td>2</td>
<td>10 enzyme loci (2.7)</td>
<td>–</td>
<td>–</td>
<td>Schuster (1989)</td>
</tr>
<tr>
<td>Pinus massoniana</td>
<td>20 830</td>
<td>10</td>
<td>9 RAPD primers</td>
<td>L &lt; M &lt; H</td>
<td>ϕ</td>
<td>Peng et al. (2003)</td>
</tr>
<tr>
<td>Pinus occarpa</td>
<td>1100 1500</td>
<td>5</td>
<td>11 isozyme loci (2.4)</td>
<td>L &lt; M &gt; H</td>
<td>×</td>
<td>Säenz-Romero &amp; Tapia-Oliviares (2003)</td>
</tr>
<tr>
<td>Pinus pallasiana</td>
<td>150 900</td>
<td>4</td>
<td>10 enzyme loci (2.9)</td>
<td>L = M = H</td>
<td>×</td>
<td>Korsikhov &amp; Mudrlik (2006)</td>
</tr>
<tr>
<td>Pinus ponderosa var.</td>
<td>1767 2579</td>
<td>3</td>
<td>7 enzyme loci (2.9)</td>
<td>L &lt; M &gt; H</td>
<td>–</td>
<td>Mitton et al. (1980)</td>
</tr>
<tr>
<td>Pinus sylvestris</td>
<td>250 1400</td>
<td>6</td>
<td>12 alloverge loci (3.7)</td>
<td>L = M = H</td>
<td>–</td>
<td>Puglisi et al. (1999)</td>
</tr>
<tr>
<td>Podocarpus parlatoei</td>
<td>1398 3100</td>
<td>18</td>
<td>25 isozyme loci (2.2)</td>
<td>L &gt; M &gt; H</td>
<td>–</td>
<td>Quiroga &amp; Premoli (2006)</td>
</tr>
<tr>
<td>Prunus mahaleb</td>
<td>1300 1635</td>
<td>7</td>
<td>12 RAPD primers (6.1)</td>
<td>L = M = H</td>
<td>x</td>
<td>Jordano &amp; Godoy (2000)</td>
</tr>
<tr>
<td>Quercus aquifolioides</td>
<td>2200 3800</td>
<td>8</td>
<td>10 isozyme loci (3.6)</td>
<td>L &lt; M &gt; H</td>
<td>–</td>
<td>Jin et al. (1998)</td>
</tr>
<tr>
<td>Quercus aquifolioides</td>
<td>2000 3600</td>
<td>5</td>
<td>6 nSSR (21.0)</td>
<td>L &lt; M &gt; H</td>
<td>Δ</td>
<td>Zhang et al. (2006)</td>
</tr>
<tr>
<td>Quercus crispula</td>
<td>850 1750</td>
<td>19</td>
<td>7 nSSR</td>
<td>L &lt; M &gt; H</td>
<td>–</td>
<td>Ohsawa et al. (2007a)</td>
</tr>
<tr>
<td>Quercus serrata</td>
<td>140 1200</td>
<td>15</td>
<td>7 nSSR</td>
<td>L = M = H</td>
<td>×</td>
<td>Ohsawa et al. (in press)</td>
</tr>
</tbody>
</table>

*For studies in which enzymes or SSRs (Simple Sequence Repeat) were used, the mean number of alleles per locus within species is shown in parentheses. For studies in which RAPDs (Random Amplified Polymorphic DNA) were used, the mean number of polymorphic bands generated by the primers is shown, and the absence of parentheses indicates that this number was not given in the cited study.
†Diversity change means the change in intrapopulation genetic diversity, generally represented by expected heterozygosity. L, M and H represent low, middle and high altitudes, respectively within each species’ range. =, < and > represent equal, increasing and decreasing diversity with increasing altitude, but there is no defined boundary between = and < or >.
‡Differentiation means interpopulation genetic variation, generally represented by fixation index or genetic distance. Vert. and Horiz. mean differentiation along vertical and horizontal axes, respectively. ϕ and × indicate that authors reported large or little differentiation, respectively. Δ indicates that there are some differences between populations, such as a cline of certain alleles or a change in rare allele number, despite little differentiation.
§The data from artificially established stands were not considered here.

occurring throughout the range expansion (Newton et al., 1999). Considering this prediction, Quiroga & Premoli (2006) suggested that such reductions in genetic diversity are consistent with evidence of patterns of forest migration, northern expansion during episodes of cooling and range contraction towards highlands during warming trends. Similarly, when the effect of introgression was removed, the number and diversity of rare alleles of Betula occidentalis decreased slightly with both elevation and latitude (the direction of migration) in a study by Williams & Arnold (2001). The loss of rare alleles in derived populations is also predicted by the range-expansion hypothesis (Williams & Arnold, 2001). Populations of Nothofagus pumilio at higher altitudes have been found to have reduced polymorphism, and these populations tended to be genetically depauperate due to the combined effects of genetic drift and/or greater inbreeding (Premoli, 2003). Again, clonal reproduction tends to be
<table>
<thead>
<tr>
<th>Species</th>
<th>Altitudinal range (m)</th>
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<th>Diversity change†</th>
<th>Differentiation‡</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabis serrata</td>
<td>1440 2400</td>
<td>10</td>
<td>7 enzyme loci (2.3)</td>
<td>(L &lt; M &gt; H) (\Delta)</td>
<td>–</td>
<td>Oyama et al. (1993)</td>
</tr>
<tr>
<td>Campanula rotundifolia</td>
<td>1500 &gt; 3000</td>
<td>19</td>
<td>4 enzyme loci (4.5)</td>
<td>(L = H)</td>
<td>×</td>
<td>Bingham &amp; Ranker (2000)</td>
</tr>
<tr>
<td>Crepis capillaris</td>
<td>45 298</td>
<td>20</td>
<td>More B-chromosomes were found at middle altitudes</td>
<td></td>
<td></td>
<td>Parker et al. (1991)</td>
</tr>
<tr>
<td>Cystopteris fragilis</td>
<td>590 1630</td>
<td>7</td>
<td>6 enzyme loci</td>
<td>(L &lt; M &lt; H)</td>
<td>(\Theta)</td>
<td>Gämperle &amp; Schneller (2002)</td>
</tr>
<tr>
<td>Dactylis glomerata</td>
<td>80 2500</td>
<td>4</td>
<td>AFLP (Amplified Fragment Length Polymorphism)</td>
<td>–</td>
<td>–</td>
<td>Reeves et al. (1998)</td>
</tr>
<tr>
<td>Dactylis glomerata</td>
<td>80 2500</td>
<td>19</td>
<td>Genome size was smaller at higher altitudes</td>
<td></td>
<td></td>
<td>Reeves et al. (1998)</td>
</tr>
<tr>
<td>Ethiopian wheat</td>
<td>1500 3300</td>
<td>133</td>
<td>29 nSSR loci (13.1)</td>
<td>(L &lt; M &gt; H)</td>
<td>–</td>
<td>Yifu et al. (2006)</td>
</tr>
<tr>
<td>Geum reptans</td>
<td>2070 3080</td>
<td>20</td>
<td>5 RAPD primers</td>
<td>(L = M = H)</td>
<td>×</td>
<td>Pluess &amp; Stocklin (2004)</td>
</tr>
<tr>
<td>Lilium longiflorum</td>
<td>800 2820</td>
<td>7</td>
<td>9 RAPD primers (7.1)</td>
<td>(L &lt; M &lt; H)</td>
<td>(\Theta)</td>
<td>Wen &amp; Hsiao (2001)</td>
</tr>
<tr>
<td>Oryza malampuzhaensis</td>
<td>110 1200</td>
<td>11</td>
<td>14 RAPD primers (1.8)</td>
<td>(L &gt; M = H)</td>
<td>(\Delta)</td>
<td>Thomas et al. (2001)</td>
</tr>
<tr>
<td>Phytolacca dodocendra</td>
<td>1600 3000</td>
<td>17</td>
<td>12 RAPD primers (5.8)</td>
<td>(L = M &gt; H)</td>
<td>(\Theta)</td>
<td>Semagn et al. (2001)</td>
</tr>
<tr>
<td>Pre-Columbian maize</td>
<td>c. 0 4000</td>
<td>193 ind.§</td>
<td>Microsatellite size was smaller at higher altitudes</td>
<td></td>
<td></td>
<td>Vigouroux et al. (2003)</td>
</tr>
<tr>
<td>Primula farinosa</td>
<td>811 1940</td>
<td>10</td>
<td>9 RAPD primers (8.8)</td>
<td>(L &lt; M &lt; H)</td>
<td>(\Theta)</td>
<td>Reisch et al. (2005)</td>
</tr>
<tr>
<td>Saxifraga oppositifolia</td>
<td>2480 3020</td>
<td>10</td>
<td>4 RAPD primers (21.0)</td>
<td>(L = H)</td>
<td>×</td>
<td>Gugerli et al. (1999)</td>
</tr>
<tr>
<td>Yushania niitakayamensis</td>
<td>1700 3200</td>
<td>13</td>
<td>9 RAPD primers (4.8)</td>
<td>(L &lt; M = H)</td>
<td>(\Theta)</td>
<td>Hsiao &amp; Lee (1999)</td>
</tr>
<tr>
<td>Sorghum bicolor</td>
<td>530 2890</td>
<td>48</td>
<td>4 enzyme loci (2.0)</td>
<td>(L &gt; M &lt; H)</td>
<td>×</td>
<td>Ayana et al. (2001)</td>
</tr>
</tbody>
</table>

*For studies in which enzymes or SSRs (Simple Sequence Repeat) were used, the mean number of alleles per locus within species is shown in parentheses. For studies in which RAPDs (Random Amplified Polymorphic DNA) were used, the mean number of polymorphic bands generated by the primers is shown, and the absence of parentheses indicates that this number was not given in the cited study.

†Diversity change means the change in intrapopulation genetic diversity, generally represented by expected heterozygosity. \(L\), \(M\) and \(H\) represent low, middle and high altitudes, respectively, within each species’ range. \(<\), \(\leq\), \(\geq\) and \(>\) represent equal, increasing and decreasing diversity with increasing altitudes, but there is no defined boundary between \(<\) and \(\leq\) or \(\geq\) and \(>\).

‡Differentiation means interpopulation genetic variation which is generally represented by fixation index or genetic distance. Vert. and Horiz. mean differentiation along vertical and horizontal axes, respectively. \(\Theta\) and × indicate that authors reported large and little differentiation, respectively. \(\Delta\) indicates that there are some differences between populations, such as a cline of certain alleles or a change in rare allele number, despite little differentiation.

§ind. means individual number.

**Figure 1** Four patterns of altitudinal changes in intrapopulation genetic diversity of plant species. \(L\), \(M\) and \(H\) represent low, middle and high altitudes, respectively. Human activities, genetic drift/bottlenecks and the harshness of the environment are the main factors affecting these patterns, and therefore the degrees of their effects are also described here.
much more common at high altitudes, which may reduce the genetic diversity in high-altitude populations. Taira et al. (1997) reported that Cryptomeria japonica changes its modes of regeneration to adapt to the local climatic conditions. It regenerates by layering only and loses genetic diversity in the forests above an altitude of 1750 m, though the largest diversity was found at intermediate altitudes (Taira et al., 1997). Therefore, the second pattern can be attributed to genetic drift and bottlenecks occurring during range expansion from lowlands, or clonal reproduction.

The third pattern, ‘L < M < H’, indicating that lower populations have reduced diversity, was found in 10 studies (24%). This pattern is found particularly in studies of herbaceous rather than tree species, and there are two main reasons for it. First, Gämperle & Schneller (2002) found greater isozyme variation and increased heterozygosity in Cystopteris fragilis populations at higher elevations than in conspecific populations at lower elevations. This result can be interpreted as indicating adaptation to more severe conditions at higher altitudes, because it is consistent with a general consensus that characteristics of individual fitness are correlated with multilocus heterozygosity (Gämperle & Schneller, 2002). However, Wen & Hsiao (2001) found larger variations in RAPD (Random Amplified Polymorphic DNA) and morphological traits in higher populations of Lilium longiflorum, suggesting that the result may be due to human factors such as collection and exploitation. Furthermore, in a survey with 435 individuals of Picea abies collected from three populations by Maghuly et al. (2006), a mitochondrial marker showed variation in just three individuals originating from the population at the highest elevation, and therefore these data suggest that the less managed high-altitude population might be autochthonous.

In addition, other factors, such as historical movements and mutation rates, may also be partially responsible for the higher diversity of populations at higher altitudes. For instance, in the Hypsithermal stage, around 4000–7000 years ago, the mean temperature was higher and the vegetation zones were 200–400 m higher than they are at present in Japan (Kaji, 1982). If plant populations were also 200–400 m higher in the Hypsithermal stage than those of the present time, and subsequently migrated downwards, relatively high genetic diversity may have been maintained in the high-altitude populations. Indeed, Ohsawa et al. (2007a) found greater diversity at high altitudes than at low altitudes, although in both cases the diversity was smaller than the diversity at intermediate altitudes. Since human activities probably do not account for genetic diversity being higher in the upper populations than the lower populations in this case, the diversity pattern identified seems to be causally linked to climate change (Ohsawa et al., 2007a).

Mutation may occur more frequently, however, at higher elevations. Although we found no genetic studies examining differences in mutation rates along altitudinal gradients in mountains, one relevant study has been conducted using a balloon. Li et al. (1997) placed dry rice and green pepper seeds in the basket of a balloon, which was then maintained at an altitude of 30–40 km for 8 hours. The seeds were then planted at ground level, and the descendents of the treated seeds were examined to determine the effects of high altitude on mutation rates. The large mutation rate found led the authors to conclude that the special conditions at high altitude could induce various mutations, most of which are heritable. Various possible reasons can be considered for the increased mutation at high altitude. For example, the level of ultraviolet-B (UV-B) radiation is higher at higher altitudes and, accordingly, Filella & Peñuelas (1999) have found increases with altitude in situ in both morphological and physiological traits that appear to provide protection against UV light in Quercus ilex and Rhododendron ferrugineum. UV-B can cause dimerization of neighbouring nucleotides, break nucleotide sequences and lead to mutations (Rozema et al., 1997). Such links have been little examined to date, so more work will be needed to understand this relationship.

The last pattern is ‘L = M = H’, which indicates that the intrapopulation genetic variation is unaffected by altitude. It was identified in the remaining 12 studies (29%) in three different situations. First, Truong et al. (2007) found no change in the genetic diversity of Betula pubescens ssp. tortuosa with increasing elevation, since the heterozygosity was similar in all of the populations they examined, but they found a large number of migrants per generation (N_e), c. 50. Thus, they concluded that high levels of gene flow compensated for possible losses in genetic diversity at high elevations and dissipated the founder effect in newly established populations above the tree line. Therefore, extensive gene flow can sometimes homogenize the distribution of genetic diversity along altitudinal gradients. Second, an ‘L = M = H’ pattern may develop if another factor affects genetic diversity more strongly than altitude. For instance, we have found no clear relationship between genetic diversity and altitude in the Quercus serrata populations we have examined (Ohsawa et al., in press). Instead, the populations exhibiting large values of expected heterozygosity are situated on water fronts, while the populations with small diversity are located on ridges and summits (Ohsawa et al., in press). Therefore, topography seems to be the main factor affecting the regeneration dynamics of the populations in this study. Finally, the last pattern may appear for reasons that are unclear. Puglisi et al. (1999) examined the genetic diversity of Pinus sylvestris populations by means of electrophoretic enzyme analysis, but they failed to detect any altitude-related trends. The cited authors suggested that this failure may have been due to the limited number of populations they sampled along the gradient and the short distances between them. However, similar results could have been obtained because of other factors, such as extensive gene flow, even if more populations had been examined.

INTERPOPULATION GENETIC VARIATION BETWEEN DIFFERENT ALTITUDES

High-altitude environments at the upper ends of strong elevational clines impose severe constraints on the reproduction and establishment of plants, due to the shortness of the growing season, low temperatures and the persistence of snow cover (Premoli, 2003; Yamagishi et al., 2005). Differences in elevation among populations also lead to marked differences in the phenology of both flowering and fruiting (Jordano & Godoy, 2000). Consequently, populations at different altitudes are sometimes
Genetic variation in plant species on mountains

differentiated from each other (Premoli, 2003; Yamagishi et al., 2005). Indeed, more than half of the previous studies of tree species and almost all the studies of herbaceous species found some evidence of differentiation (Tables 1 & 2).

Such differentiation can occur at several different levels: chromosome, genome size or just a few loci. At the chromosomal level, the variation in B-chromosomes (Bs) is well known: these are ‘additional dispensable chromosomes that are present in some individuals from some populations in some species, which have probably arisen from A-chromosomes but follow their own evolutionary pathway’ (Beukeboom, 1994). Parker et al. (1991) found that frequencies of Bs of Crepis capillaris were maximal at intermediate altitudes, suggesting that Bs in Crepis are excluded from populations growing under suboptimal conditions. However, the reason for this finding is still unclear.

Genome size is also correlated with altitude. Using microdensitometry, Reeves et al. (1998) found a significant negative correlation between genome size and altitude in natural populations of Dactylis glomerata. Species with small genomes tend to have more rapid rates of replication of nuclear DNA and cell division than species with larger genomes (Reeves et al., 1998). Taking this into account, Reeves et al. (1998) proposed that the harsher climates and shorter growing seasons at high altitudes may favour genotypes of D. glomerata with smaller genomes, the meristems of which have larger proportions of rapidly cycling cells than lower-altitude genotypes with larger genomes. In addition, Vigouroux et al. (2003) analysed 99 microsatellite loci in a sample of 193 maize plants representing the entire pre-Columbian range of this crop, and observed a negative correlation between allele size and altitude: an average decrease of 1.8 base pairs (bp) per locus per 1000 m elevation (Vigouroux et al., 2003). The reason for this finding is still unclear, but two possible explanations were proposed. First, they suggested that selection pressures may favour a smaller genome in short-season environments, as described above. Second, at higher elevations, maize has a shorter generation time (fewer cell divisions) and thus is likely to have smaller mutation rates per generation. Thus, since mutations tend to increase in the size of microsatellites, a smaller mutation rate at high altitudes could result in smaller average allele sizes (Vigouroux et al., 2003). Irrespective of which explanation is true, these changes in chromosome or genome size remain little reported for tree species, probably because the rate of differentiation and evolution are slower for trees than for herbaceous species. Nevertheless, we recommend that researchers, even those working on tree species, examine not only values of genetic differentiation, such as $F_{ST}$, but also changes in genome or allele size between populations at different altitudes.

At the locus level, a particular locus or particular allele at a certain locus may show clear differentiation between populations at different altitudes as a result of natural selection. Using amplified fragment length polymorphism (AFLP) analysis, Jump et al. (2006) reported that, at its upper and lower altitudinal limits, Fagus sylvatica is exceptionally differentiated at a particular locus. Differentiation at this locus is significantly greater than that expected assuming selective neutrality, suggesting that a region of the F. sylvatica genome is strongly subject to natural selective pressures operating between the upper and lower limits of the species’ distribution (Jump et al., 2006). Highly heterogeneous environmental conditions imposed by altitudinal gradients are likely to affect the neutral sites closely linked to the site under selection (Zhang et al., 2006).

Even in other regions or sites in genomes that are unaffected, or weakly affected, by natural selection, genetic drift can sometimes cause differentiation between populations at different altitudes. To detect such differentiation, analysis of molecular variance (AMOVA) as described by Excoffier et al. (1992) has often been used, because it allows us to partition genetic variation into multiple components, such as among groups, among populations within groups and among individuals within populations. Reisch et al. (2005) observed a molecular variance of 8.47% between the two altitudinal groups of Primula farinosa populations above and below 1750 m. Again, genetic distances ($\Phi_{ST}$) were significantly correlated not with horizontal distances but with the altitudinal distances between populations (Reisch et al., 2005). This altitudinal differentiation is ascribed to different flowering times of upper and lower populations and to the forests beneath the tree line, which is between upper and lower populations (Reisch et al., 2005).

In contrast to these studies reporting differentiation, several studies have found no differentiation between populations at low and high altitudes. According to the results of an AMOVA conducted by Ohsawa et al. (2007a), the amount of genetic variance of Quercus crispula partitioned among altitudinal groups and among populations within groups was 0.04% and 1.39%, respectively, with the remaining variance (98.55%) occurring within populations. This result can be explained by two main factors. Overlap of flowering phenology in populations at different altitudes, in combination with the species’ extensive pollen flow, may be one of the factors limiting differentiation (Ohsawa et al., 2007a). Long-distance seed dispersal between different altitudes by animals, particularly birds, may be another contributory factor for the lack of differentiation (Ohsawa et al., 2007a). Lack of differentiation may also be due to short population histories in some cases. For instance, Alden & Loopstra (1987) found no strong population substructure for Picea glauca along an altitudinal gradient in the interior of Alaska, and suggested that there has been insufficient time for evolutionary forces to differentiate populations because the subarctic climate of interior Alaska has rapidly changed. Populations on the upper slopes and at the tree limit may have diverged only about 2500 years ago, and reached tree-limit altitudes only recently (Alden & Loopstra, 1987). In addition to extensive gene flow and a short population history, a large effective population size may be another contributory factor to low levels of differentiation between populations at different altitudes (Muir et al., 2004). Therefore, when no altitudinal differentiation is apparent, all these factors must be taken into account to determine the reasons for its absence.

Finally, genetic structures along altitudinal gradients may also be influenced by interspecific hybridization, although no case studies of hybridization are shown in Tables 1 & 2. Few previous studies have reported such effects, but we can easily imagine them in the field. Populations of one species often occur adjacent
to closely related species within different altitudinal ranges, and they coincide at the boundaries. Consequently, alleles of some species can flow into other species, causing a genetic cline at particular loci along altitudinal gradients. Senjo et al. (1999) found that paternal chloroplast DNA flows from *Pinus parviflora* var. *pentaphylla* at lower altitudes to *Pinus pumila*, and that maternal mitochondrial DNA flows from *P. pumila* at higher altitudes to *P. parviflora* var. *pentaphylla*. Such phenomena may also affect the distribution of intrapopulation genetic diversity, because the inflow of new alleles can increase allelic richness.

**INTERPOPULATION PHENOTYPIC VARIATION BETWEEN DIFFERENT ALTITUDES**

There have been many studies on interpopulation phenotypic variation between different altitudes in common garden experiments over the last few decades (e.g. Hamrick, 1976). Here, we consider only some of them conducted in common garden experiments with seeds collected from several sites (Table 3), and therefore the results can be attributed to genetic factors but not to environmental factors. Several (Isik & Kara, 1997; Sáenz-Romero et al., 2003) studied isoenzyme variations in *Pinus oocarpa* populations, which indicated that there are intense gene flows among populations, and all the populations could be considered a single panmictic population. Nevertheless, Sáenz-Romero et al. (2006) found significant differences among the same populations in terms of growth traits, concluding that the selectively neutral isoenzymes are not capable of expressing the genetic differentiation among *P. oocarpa* populations. Such a conclusion highlights the importance of phenotypic examinations in detecting the effect of natural selection on altitudinal differentiation.

### Table 3 Published case studies on altitudinal phenotypic variation in plant species in common garden experiments with the seeds collected from various sites.

<table>
<thead>
<tr>
<th>Species</th>
<th>Altitudinal range (m)</th>
<th>Population number</th>
<th>Main examined traits/examination method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Abies concolor</em></td>
<td>1280–2073</td>
<td>4</td>
<td>Provenance test*</td>
<td>Hamrick (1976)</td>
</tr>
<tr>
<td><em>Abies sachalinensis</em></td>
<td>600–1200</td>
<td>3 + 3</td>
<td>Freezing resistance†</td>
<td>Eiga &amp; Sakai (1984)</td>
</tr>
<tr>
<td><em>Pinus brutia</em></td>
<td>61–1033</td>
<td>10</td>
<td>Provenance test‡</td>
<td>Isik &amp; Kara (1997)</td>
</tr>
<tr>
<td><em>Pinus oocarpa</em></td>
<td>1075–1505</td>
<td>5</td>
<td>Provenance test§</td>
<td>Sáenz-Romero et al. (2006)</td>
</tr>
</tbody>
</table>

*High-elevation population samples were smaller in size and needle measurements, had fewer adaxial stomal rows, blunter needle tips, and a shorter growing season.

†Freezing resistance of winter buds of the open-pollinated progenies from 51 mother trees and ramets from 80 cones, growing at different altitudes, were assessed. Freezing resistance increased with increasing altitude.

‡Six-year-old seedlings from middle-elevation populations had better performance and better uniformity. They also exhibited higher adaptational plasticity and higher stability under varying environmental conditions.

§Seedlings from lower altitudes tended to grow better, because they were adapted to the milder climates. The authors suggest guidelines based on delimitation of three altitudinal seed zones of about 200 m in breadth.

The most widely known variation of phenotype along altitudinal gradients is in growth traits such as tree size. Growth traits often have stronger selection pressures acting on them than other traits, producing more differentiation among populations (Hamrick, 1976). Although individuals collected from intermediate elevations exhibit stronger growth in some species, as mentioned above, tree size generally decreases with increasing altitude (Shibata, 1985; Sakai et al., 2003). This may be partly because of environmental variation, but also partly because of genetic variation (Shibata, 1985). Sakai et al. (2003) proposed that life-history adaptation, i.e. earlier resource allocation to reproduction at higher sites, is one of the reasons why trees are smaller at higher altitudes. Irrespective of whether this hypothesis is true, the life span of plant species tends to decline with increasing altitude (Sakai et al., 2003; Vigouroux et al., 2003). Such shortened life cycles may accelerate the speed of adaptive evolution at high altitudes, and thus promote altitudinal differentiation. Smaller trees can probably disperse their pollen and/or seeds over shorter distances than taller ones, promoting interpopulation differentiation.

**GENETIC CHANGES ACROSS MOUNTAINS ALONG HORIZONTAL AXES**

The simplest explanation for genetic variations among populations that are located at sites with similar altitudes, but separated by mountainous topography, is that they were caused by barriers to seed-mediated genetic exchange between populations located on opposite sides of ridges. However, empirical case studies of such barriers, with respect to plant species, are still limited. Reviewing previous reports, Taberlet et al. (1998) analysed the phylegeographies of several taxa in order to elucidate the amount and distribution of genetic variation across Europe. They found
that Italian lineages were often isolated due to the presence of the Alps as a barrier, suggesting that Italy has many endemic lineages. Cottrell et al. (2005) recently examined variation of chloroplast DNA in black poplar (Populus nigra) across Europe, and identified the presence of a specific haplotype throughout Italy and Austria which was almost entirely absent from France. This pattern of distribution suggests that the Alps formed a very effective barrier to the movement of this haplotype into France, but appeared to allow migration into Austria (Cottrell et al., 2005). Similarly, studies of variation of chloroplast DNA have demonstrated that the central mountain ridge of Taiwan creates an insurmountable barrier to the east–west gene flow of Cyclobalanopsis glauca and Castanopsis carlesii (Huang et al., 2002; Cheng et al., 2005). In this way, genetic differentiation has often occurred across mountains along not only vertical but also horizontal axes.

If gene flow via seed is thus interrupted across ridges, genetic diversity can also vary between one side of a mountain and the other. Assuming that, for example, a tree population has survived a glacial period on the southern side of high ridges and then migrated northwards in the post-glacial period, the populations on the northern side may have lost genetic variation due to genetic drift and/or bottleneck effects. Indeed, much greater genetic diversity in the southern areas of the Alps than in recently colonized northern regions has been documented for several species in Italy (Taberlet et al., 1998). In the near future, such changes in genetic variation need to be assessed along horizontal axes in other mountain areas.

Similarly, areas where refugia have been postulated, such as the Iberian and Italian peninsulas, generally displayed higher levels of chloroplast diversity in oak (Quercus) species (Petit et al., 2002). Petit et al. (2002) reported that most chloroplast haplotypes of Quercus found in northern Europe are also present in the south, whereas the converse is not true, suggesting that the majority of mutations observed were generated prior to post-glacial recolonization. Therefore, the Alps formed a partial barrier even to the movement of Quercus. Nevertheless, several lineages of some genera, such as Abies and Quercus, were able to cross even the Alps and to spread to the north, east and west (Taberlet et al., 1998). Moreover, the central mountain ridge of Taiwan did not act as a barrier to Trochodendron aralioides, the distribution of which is centred on high elevations (Huang et al., 2004). To cross ridges, seeds must ascend and then descend slopes, so vertical seed flow is clearly needed. For example, nutcrackers (Nucifraga caryocatactes) often transport seeds of beech (Fagus crenata), allowing F. crenata to migrate into alpine zones or other high-altitude areas (Watanabe, 1994). Such long-distance vertical seed flow between different elevations is important for plant gene flow across ridges, but few studies have addressed this issue. In addition, short-distance but frequent seed flow may also play a pivotal role in species ascending or descending slopes. Some recent studies of genetic structures have highlighted the importance of local seed dispersal processes to explain the colonization of trees in response to climatic warming (Pearson, 2006). Ohsawa et al. (2007b) tracked seed flow of Q. crispula at a fine scale on various slopes, identifying the mother tree of each seedling examined based on nuclear genetic information obtained from endocarps of hypogal cotyledons attached to the seedlings. They found that most seeds were dispersed downwards, but evidence of upward dispersal was also detected, even on steep slopes. Further work is required on seed flow on mountains to evaluate the potential for individual tree species to cross ridges.

A recent study using palaeobotanical and genetic data for European beech (F. sylvatica) found that the mountain chains in Europe were not geographical barriers to this species but rather facilitated its dispersal (Magri et al., 2006). Conversely, its spread was hindered by large plains or valleys (Magri et al., 2006). Such phenomena can occur particularly in species that are confined to highland environments. Therefore, the role of ridges in gene flow, migrations and associated phenomena may differ depending on the species’ altitudinal distribution, but the steepness of the mountains and/or the dispersal mode of the species’ seed may also influence these processes.

CONCLUSIONS

In conclusion, we consider the possible interpretations and implications of the patterns of genetic variation on mountains described above.

Conclusions from the review of previous studies

Genetic diversity within populations can vary along altitudinal gradients as a result of various factors. Twenty-six per cent of total case studies showed the well known pattern – that the populations at lower and higher elevations have relatively small diversity compared with the populations at intermediate elevations – but other patterns have also been observed in many of the studies considered. Thus, geographically core populations are not always the most diverse. Further, some authors have found no relationship between genetic diversity and altitude. So altitude is not always a useful variable when estimating the genetic diversity of plant populations in mountainous regions. To determine which population has the largest diversity, it is necessary to consider not only altitude but also the physiological characteristics of each species and the history of its populations.

In the examined studies, there was evidence of altitudinal differentiation in half of those pertaining to either tree species or herbaceous species. Even in the cases where no differentiation was identified, intrapopulation genetic variation often changed along altitudinal gradients. Further, phenotypic variation between populations at different altitudes was detected in some studies, but there was no associated variation in molecular data. These results suggest that some differentiation and/or some intrapopulation variation often exist across different altitudes. Therefore, such altitudinal influences should be taken into account when genetic diversity and/or the structure of tree species are investigated in mountainous areas. In this respect, it is problematic that some previous studies have not taken into account the altitude of the investigated populations. In addition, natural seeds and seedlings from specific altitudes should not be transplanted to different altitudes, even if molecular markers do not indicate differentiation. To some extent, however, zoning
seed sources is possible. As discussed above, examining phenotypic traits can help to identify altitudinal differentiation caused not only by mutation, genetic drift and gene flow but also by natural selection. However, provenance tests would take a long time, especially for tree species. Thus, molecular markers are useful as an interim method for determining zones, though neutral markers cannot reflect the effect of natural selection.

Moreover, genetic differentiation can also occur across mountains along horizontal axes, in tree species that occur at relatively low altitudes. However, ridges can provide pathways rather than barriers for high-altitude tree species.

A unified model based on both vertical and horizontal patterns

Populations with unique genetic structures on mountains are likely to change as a result of global warming. A number of studies have suggested that global warming may cause shifts in the distribution of plant species in high-mountain ecosystems (Gottfried et al., 1999). Furthermore, Grabherr et al. (1994) found that the species richness of vascular plants has already increased in recent decades on summits in the Alps. They concluded that there is an overall tendency for the alpine-nival flora to move upwards. Slatyer & Noble (1992) suggested that the tree line may not represent an absolute limit to growth, but rather a narrow zone in which the establishment of seedlings above the adult tree line is relatively common but balanced by periods of catastrophic mortality caused by disturbance or severe weather. Forest management must now take account of such global changes.

In terms of this issue, a likely scenario based on both vertical and horizontal patterns of genetic diversity and structure would be helpful in order to understand the global distribution and migration of plant species. Here, we describe a likely scenario based on some patterns identified in this paper (Fig. 2). At low latitudes, plant species tend to occur in some limited areas on ridges and summits at relatively high altitudes. Considering the effect of global warming, areas at low altitudes are likely to provide suboptimal environments for many species, resulting in a ‘L < M < H’ pattern. In such cases, ridges may provide important habitats and pathways, as reported by Magri et al. (2006). Species thus affected need to be conserved immediately. Individuals at higher altitudes are unlikely to find places to escape from warming, and are therefore likely to disappear in the near future. To conserve these species, the populations on different ridges should be conserved separately, as they represent scattered islands. At middle latitudes, many species may exhibit ‘L < M > H’ patterns. Whether or not a ridge is likely to impose a genetic barrier for any given species depends on the elevations of its distribution and other factors. However, altitudinal zoning is needed to maintain the genetic resources and structures of such species. At high latitudes, many plant species may, currently, never reach high altitudes. Hence, the ‘L > M > H’ pattern is common, and ridges can be genetic barriers for many plant species. Such ridges can and should be treated as boundaries partitioning conservation units.

The importance of isolation by mountainous topographies in terms of evolution

Finally, the separation and partial isolation of populations imposed by mountainous topographies along both vertical and horizontal axes has resulted in genetic structures, with important
evolutionary implications. There are two types of isolation: with and without environmental differences. If there are environmental differences between patches or demes, natural selection occurs on non-neutral loci in each patch according to its micro-environment. Consequently, differentiation and speciation between patches occurs, thus increasing species diversity. Altitudinal isolation is a typical case of this type of isolation, because many abiotic and biotic factors vary with altitude. Further, isolation along horizontal axes by ridges lying west–east may also be associated with the first type of isolation, because the southern and northern sides of these ridges may provide contrasting environments (e.g. warm and cool). The second type of isolation, without marked environmental differences, can present favourable conditions for the evolution of species. Wright's (1931) shifting balance theory suggests that subdivision of a large population into partially isolated subpopulations enhances evolution on non-neutral loci through a three-phase process: random drift, intrademe selection and interdeme selection. Isolation along a horizontal axis by ridges lying north–south may represent the second type of isolation. Although Wright's theory is still under discussion, isolation by mountains probably plays an important role in diversification and evolution.

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REFERENCES


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