

# Chapter 4

## Plant Genetic Diversity and Plant–Pollinator Interactions Along Altitudinal Gradients

Takao Itino and Akira S. Hirao

**Abstract** Alpine plants are thought to be particularly vulnerable to extinction as a result of global warming because their habitat ranges are expected to shift upward until, eventually, no higher habitats remain into which they can escape. Moreover, even mountain plants distributing across wide altitudinal ranges are likely to experience range shifts, raising the possibility of local extinctions, especially of genetically and ecologically differentiated types with narrow distribution ranges. In this chapter, we examine to what extent plants genetically and ecologically differentiate along altitude and how altitudinal changes in pollinator assemblages affect floral evolution. First, by literature survey, we demonstrate that although altitudinal patterns of species and genetic diversity have been extensively investigated, few clear-cut examples of altitudinal genetic differentiation of neutral markers have been identified. On the other hand, many studies have shown that adaptive traits of plants differentiate along altitude, although their adaptive genetic background is yet to be uncovered. We then briefly introduce three case studies of mountain herbaceous plant species displaying cryptic neutral genetic or adaptive trait differentiation. The first two case studies show that the floral size of both *Campanula punctata* and *Prunella vulgaris* differs among populations along altitude and is adapted to altitudinally variable pollinator size. The third case study demonstrates that *Cimicifuga simplex* is composed of three genetically differentiated ecotypes that are distributed parapatrically or allopatrically along an altitudinal gradient. To meet the challenges posed by future climate warming, it is essential to clarify the genetic and ecological differentiation of mountain plants along altitude.

**Keywords** Alpine plants • Bumblebees • Climate warming • Conservation unit • Floral size • Species interaction

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T. Itino (✉)

Department of Biology, Faculty of Science, Shinshu University, Nagano, Japan

Institute of Mountain Science, Shinshu University, Nagano, Japan

e-mail: [itino@shinshu-u.ac.jp](mailto:itino@shinshu-u.ac.jp)

A.S. Hirao

Sugadaira Montane Research Center, University of Tsukuba, Tsukuba, Japan

## 29 4.1 Introduction

30 High mountain plants are expected to react sensitively to climate warming. In  
31 particular, plants living near the top of elevation gradients are expected to become  
32 extinct as plant species shift upward under a warming climate (Theurillat and  
33 Guisan 2001; Colwell et al. 2008). Consequently, a reduction of biodiversity in  
34 alpine habitats is predicted (Chen et al. 2011). Results from a standardized moni-  
35 toring network of 60 sites on European mountain summits support these inferences:  
36 Pauli et al. (2012) reported that between 2001 and 2008, species moved upslope on  
37 average, and Gottfried et al. (2012) presented evidence for a decline in cold-adapted  
38 species and an increase in warm-adapted species at mountain summits.

39 Most studies, however, have focused on species- and community-level effects of  
40 climate warming, and few studies have examined intraspecific diversity (Pauls  
41 et al. 2013). Because taxonomically recognized morphospecies are often not evolu-  
42 tionarily or ecologically relevant units (Bálint et al. 2011), morphospecies-based  
43 approaches may seriously underestimate the effects of climate warming on biodi-  
44 versity. Therefore, accurate assessment of such effects requires identification of  
45 cryptic intraspecific differentiation (Bálint et al. 2011; Pauls et al. 2013).

46 In mountain ranges, steep environmental clines along altitude can strongly  
47 influence adaptive traits and, potentially, lead to adaptive diversification within a  
48 narrow geographic range (Byars et al. 2007; Milá et al. 2009). This adaptive  
49 diversification may in turn lead to genetic differentiation, especially when adapta-  
50 tion of the reproductive organs is involved. Thus, mountain ecosystems are ideal for  
51 exploring adaptive and genetic differentiation over short distances. For a full  
52 understanding of mountain biodiversity and how to conserve it in the face of  
53 climate change, elucidation of altitudinal differentiation between populations living  
54 at higher and lower elevations is essential.

55 In this chapter, we examine to what extent plants genetically and ecologically  
56 differentiate along altitude and how altitudinal changes in pollinator assemblages  
57 affect adaptive differentiation of the floral traits of plants. We first present a  
58 literature overview, and then we examine the details in some case studies. We  
59 then propose a framework for the conservation of mountain plants. Our literature  
60 survey, presented in Sect. 4.2, shows that, despite numerous investigations of  
61 altitudinal patterns of species and genetic diversity, few clear-cut examples of  
62 altitudinal genetic differentiation of neutral markers in plants have been identified.  
63 However, numerous studies have reported adaptive phenotypic divergence along  
64 altitude, and recently, some pioneering eco-genomic studies have examined the  
65 genetic background of such altitudinal adaptive diversification. In Sect. 4.3, we  
66 introduce three case studies of cryptic genetic and ecological differentiation of  
67 mountain herbaceous plant species that inhabit a wide altitudinal range in the  
68 mountains of central Japan. The cases of *Campanula punctata* var. *hondoensis*  
69 (case 1) and *Prunella vulgaris* (case 2) show that the floral size of these species  
70 differs among populations along altitude, and they are locally adapted to  
71 altitudinally variable pollinators of different size. The third case study demonstrates

that in central Japan, *Cimicifuga simplex* comprises at least three genetically differentiated ecotypes that are distributed parapatrically or allopatrically along altitudinal gradients.

## 4.2 Global Pattern of Biodiversity Along Altitudinal Gradients

### 4.2.1 Altitudinal Gradients in Ecological Research

Altitudinal gradients in biodiversity are nearly as ubiquitous as latitudinal gradients, and they have some characteristics that make them, perhaps, more suitable for uncovering the causes underlying the ecological and evolutionary responses of biota to such gradients (Körner 2007). First, changes in ecological phenomena occur over a shorter distance along an altitudinal gradient than along a latitudinal gradient, because temperature often decreases rapidly, by as much as 5.5–6.5 °C per 1000 m, with increasing altitude. In contrast, a comparable temperature decline occurs over ~1000 km of latitude (Jump et al. 2009). Thus, field data can be collected more readily along altitudinal gradients than along latitudinal gradients simply because the spatial extent of an altitudinal gradient is small compared to that of a latitudinal gradient. Second, it is possible to study many replicates of altitudinal diversity gradients, because each mountain or mountain range is a potential replicate, and to thereby assess the generality of the causes underlying the responses of biota. Third, manipulative experiments such as reciprocal transplants along altitudinal gradients can test theories of evolutionary adaptation over short distances.

Environmental factors are either physically linked to altitude, such as land area and climatic variables such as temperature, atmospheric pressure, and UV radiation, or they are correlated with altitude, such as vegetation zones and land use (Körner 2003, 2007). Thus, a suite of environmental gradients accompanies changes in altitude. By exploring an organism's response to this suite of co-varying factors, ecological researchers have investigated the underlying causes of biodiversity. In this section, we present a literature review of patterns of plant biodiversity along altitude at the species and gene level.

### 4.2.2 Species Diversity Along Altitudinal Gradients

Altitudinal trends in species biodiversity are generally thought to mirror latitudinal trends, with biodiversity decreasing both at higher altitudes and higher latitudes. Consistent with this assumption, some studies have found a decreasing trend in plant species richness with altitude and have attributed this plant diversity trend to corresponding gradients in climate and primary productivity (Kitayama 1992;

107 Odland and Birks 1999; Grytnes and Vetaas 2002; Grytnes 2003). However,  
108 species biodiversity does not always show a decreasing trend with altitude. Rahbek  
109 (1995) reviewed biodiversity patterns in relation to altitude and showed that the  
110 prevailing pattern is for species richness to peak at an intermediate altitude. The  
111 occurrence of peak diversity at intermediate altitudes has since been reported across  
112 a wide range of taxa (Rowe 2009; Wang et al. 2009; reviewed in Sanders and  
113 Rahbek 2012). Notably, in alpine regions, either diversity decreases along altitudi-  
114 nal gradients or the diversity gradient has a humped shape, with peak diversity at  
115 intermediate altitude (Rahbek 1995; Grytnes 2003; Nogues-Bravo et al. 2008). In  
116 addition, anthropogenic factors such as grazing can disturb natural altitudinal  
117 diversity patterns (Speed et al. 2013).

118 The occurrence of a humped relationship between species richness and altitude  
119 is presumably attributable to complex factors such as climate and productivity,  
120 geographic constraints on the ranges of species, the disturbance history of the  
121 landscape, and the evolutionary history of the species (Grytnes and Vetaas 2002).  
122 In addition, altitudinal gradients in the phylogenetic structure of communities can  
123 reflect the interplay of biotic and abiotic constraints on diversity (Machac  
124 et al. 2011). For example, Shrestha et al. (2014) reported significant phylogenetic  
125 clustering of flowering plant communities along an altitudinal gradient with phy-  
126 logenetically related species showing more variety in flower color than predicted by  
127 the null evolution model. Evolutionary history may also influence the mix of  
128 species that contributes to the diversity and structure of biotic communities  
129 (Webb et al. 2002).

### 130 **4.2.3 Altitudinal Patterns of Genetic Diversity**

131 The causes underlying intraspecific genetic diversity can also be explored along  
132 altitudinal gradients. A steep, continuous, altitudinal gradient can maximize both  
133 environmental differences and the potential for gene flow, thus providing opportu-  
134 nities for studying the relative effects of gene flow and natural selection on spatial  
135 patterns of genetic variation in populations. Altitudinal gradients have already been  
136 used by evolutionary biologists to obtain evidence for population differentiation in  
137 response to environmental change (Clausen et al. 1940; Meinzer et al. 1985;  
138 Gurevitch 1988; Galen et al. 1991). The application of molecular markers to the  
139 measurement of genetic variation has revealed extremely complex distributional  
140 patterns of genetic variation within and among populations along altitudinal gradi-  
141 ents that are highly variable among species (Herrera and Bazaga 2008; Byars  
142 et al. 2009; Hahn et al. 2012).

143 After reviewing published studies of 48 plant species, Ohsawa and Ide (2008)  
144 reported that genetic variation along altitudinal gradients on mountains is generally  
145 categorized and explained as follows. First, intermediate-altitude populations  
146 sometimes have higher levels of genetic variation than either low- or high-altitude  
147 populations because, in accordance with the central–marginal hypothesis (Eckert

et al. 2008), habitats at intermediate altitude are optimal. Low- and high-altitude populations tend to occur near the outer boundaries of a species' range, where limitations on gene flow, population size, and founder effects all promote genetic drift, thereby reducing genetic variation and increasing differentiation among populations. Second, low-altitude populations may have the highest level of genetic diversity, which decreases with altitude probably because optimal low-altitude habitats and founder effect through upward expansion occur. Third, the highest genetic diversity may occur at high altitudes because of decreased human disturbance, a historical downward range shift due to climate change, or adaptation. Lastly, genetic variation is sometimes largely constant along a given altitudinal gradient because of extensive gene flow between populations.

Overall, variations in the genetic drift–gene flow balance along altitudinal gradients are expected to result in altitudinal variations in the genetic structures and diversity of populations. However, other factors potentially influence the genetic diversity of populations on mountains. First, biogeographic history affects the genetic diversity of populations. At a large spatial scale in Europe, the genetic structure of populations of mountainous species mainly reflects historical factors such as the locations of glacial refugia and subsequent recolonization during postglacial expansion (Hewitt 2000; Petit et al. 2003). For example, genetic diversity of high-mountain plants in the Alps and the Carpathians does not reflect altitude, and it differs between these two mountain systems because of their divergent phylogeographic history (Thiel-Egenter et al. 2009). In contrast to Europe, where continental glaciation occurred during the Quaternary, far eastern Asia, including the Japanese archipelago, was not covered by a continuous ice sheet during the Pleistocene (Frenzel 1968; Svendsen et al. 2004), and several studies have documented the resulting distinctive biogeographic history (Fujii and Senni 2006; Ohsawa and Ide 2011; Hirao et al. 2015). Life history traits have also long been hypothesized to influence genetic diversity (Hamrick and Godt 1989; Nybom and Bartish 2000), and significant changes in life history traits such as the breeding system, the pollen and seed dispersal mode, and successional status along altitude have the potential to influence the genetic diversity of populations. For example, it has been suggested that the presence of fewer and less active pollinators at high compared with low altitude (Arroyo et al. 1985; Bingham and Orthner 1998; Malo and Baonza 2002) leads to clonality and selfing in plants at high altitude (Jacquemyn et al. 2005 but see also Wirth et al. 2010). Because inbreeding is well known to reduce genetic diversity, these life history trait changes might cause plant species to experience a decrease in genetic diversity along altitudinal gradients. The processes underlying these patterns can be either neutral, such as genetic drift and bottleneck effects due to demographic history, or selective, if they are caused by environmental clines related to altitude changes.

#### 188 4.2.4 *Altitudinal Genetic Differentiation of Neutral Markers*

189 Numerous studies of mountain plants have found significant genetic differentiation  
190 and structuring among plant populations along altitudinal gradients (Ohsawa and  
191 Ide 2008; Byars et al. 2009; Shi et al. 2011). However, studies attempting to  
192 quantify the degree of genetic differentiation along altitude are relatively scarce.  
193 To consider differential patterns of population genetic structures along altitudinal  
194 gradients, we reviewed published studies that investigated altitudinal differentia-  
195 tion of neutral molecular markers. In all, we found 25 papers that examined a total  
196 of 26 species (Table 4.1), but few of these studies demonstrated clear-cut altitudinal  
197 genetic differentiation in plant populations. In most cases, the genetic variance  
198 component explained by altitudinal differences was subtle or weak, ranging from  
199 none to 10 % with a mean of 3.1 %. This result is somewhat counterintuitive,  
200 because one would expect many mountainous species to exhibit phenological  
201 differences along altitudinal gradients (Dittmar and Elling 2006; Ziello  
202 et al. 2009). For example, altitudinal differences in the timing of flowering should  
203 promote reproductive isolation and, thus, genetic differentiation along altitude.  
204 Gene flow across altitudinal gradients hindering complete reproductive isolation  
205 among populations inhabiting different altitudes might, however, explain the  
206 reported subtle or weak altitudinal differentiation (e.g., Hahn et al. 2012). In fact,  
207 Matter et al. (2013) demonstrated that contemporary pollen-mediated gene flow  
208 between populations occurs in two herb species across an altitudinal gradient.  
209 Similarly, effective gene flow across an altitudinal gradient has been shown to  
210 explain the high levels of genetic diversity found in the alpine species *Campanula*  
211 *rotundifolia* (Bingham and Ranker 2000) and *Arabis serrata* (Okuyama et al. 2012).  
212 In mountain regions, levels of gene flow between populations appear to be highly  
213 variable. In some species, restricted gene flow ( $N_e m < 1$ , where  $N_e$  is population size  
214 and  $m$  is the fraction of migrants) has been detected between altitudinal populations  
215 (Barbara et al. 2007), whereas in other species a moderate level of gene flow ( $N_e m$   
216  $\geq \sim 1$ ) has been found (e.g., Byars et al. 2009). A paternity analysis of *Arabis alpina*  
217 in an alpine landscape revealed that several long-distance ( $\geq 1$  km) pollen flow  
218 events had occurred (Buehler et al. 2012). Garcia-Fernandez et al. (2012) also  
219 reported a substantial level of gene flow within altitudinal gradients and suggested  
220 that seed and, especially, pollen dispersal is more likely to occur between  
221 populations along an altitudinal gradient on the same mountain than between  
222 populations located at the same altitude on different mountains, although occa-  
223 sional horizontal movement cannot be ruled out. Overall, these results indicate that  
224 several populations distributed across altitudinal gradients on a mountain or in a  
225 mountain range can form a metapopulation with a single genetic structure.

226 Sexton et al. (2014) have proposed that gene flow follows one of three patterns  
227 with respect to spatial and environmental gradients. Gene flow may be higher  
228 between geographically close populations, between populations in similar environ-  
229 ments, or between populations in dissimilar environments (counter-gradient flow).  
230 If the gene flow rate is higher among populations at similar altitudes rather than

t1.1 **Table 4.1** Published studies of altitudinal genetic differences in plant populations

t1.2	Species	Life form	Altitudinal range (m)	Area (km <sup>2</sup> )	No. populations	Overall $F_{ST}$ or $F_{ST}$ analogue	Variance component of genetic variation explained by altitudinal differences	Marker type	Reference
t1.3	<i>Athyrium filix-femina</i>	Fern	450–1800	8000	20	0.040	0.001	RAPD	Schneller and Liebst (2007)
t1.4	<i>Briza media</i>	Forb	1200–1800	22,000	20	0.099	0.059	AFLP	Hahn et al. (2012)
t1.5	<i>Geum reptans</i>	Forb	2070–3080	12,500	20	0.150	0.024	RAPD	Pluess and Stöcklin (2004)
t1.6	<i>Isodon umbrosus</i>	Forb	320–1730	62,500	15	0.360	–	Isozymes	Dohzono and Suzuki (2010)
t1.7	<i>Lilium longiflorum</i>	Forb	720–2820	9100	7	0.066	0.051	RAPD	Wen and Hsiao (2001)
t1.8	<i>Polemonium viscosum</i>	Forb	3500–4000	–	2	0.015–0.069	0.015–0.069	Isozymes	Galen et al. (1991)
t1.9	<i>Primula farinosa</i>	Forb	811–1940	32	10	0.170	0.085	RAPD	Reisch et al. (2005)
t1.10	<i>Ranunculus bulbosus</i>	Forb	1200–1800	14,000	18	0.071	0.043	AFLP	Hahn et al. (2012)
t1.11	<i>Saxifraga oppositifolia</i>	Forb	2480–3020	2500	10	0.04–0.05	0.000	RAPD	Gugerli et al. (1999)
t1.12	<i>Trifolium montanum</i>	Forb	1200–1800	22,000	20	0.118	0.054	AFLP	Hahn et al. (2012)
t1.13	<i>Trifolium montanum</i>	Forb	682–2066	600	61	0.044	–	AFLP	Hahn et al. (2013)
t1.14	<i>Festuca eskia</i>	Grass	1582–2604	20,000	9	0.380	0.040	Microsatellites	Gonzalo-Turpin and Hazard (2009)

(continued)

t1.15 **Table 4.1** (continued)

Species	Life form	Altitudinal range (m)		Area (km <sup>2</sup> )	No. populations	Overall $F_{ST}$ or $F_{ST}$ analogue	Variance component of genetic variation explained by altitudinal differences	Marker type	Reference
t1.16 <i>Hordeum vulgare</i>	Grass	1798	3324	720,000	106	0.105	0.100	Microsatellites	Hadado et al. (2010)
t1.17 <i>Poa hiemata</i>	Grass	1700	1880	180	11	0.096	0.045	Microsatellites	Byars et al. (2009)
t1.18 <i>Sorghum bicolor</i>	Grass	530	2890	1,248,000	48	0.410	0.030	Isozymes	Ayana et al. (2001)
t1.19 <i>Hippophae rhamnoides</i>	Shrub	1800	3400	80,000	5	0.097	–	Microsatellites	Chen et al. (2008)
t1.20 <i>Lavandula latifolia</i>	Shrub	990	1540	22,500	21	0.298	0.000	AFLP	Herrera and Bazaga (2008)
t1.21 <i>Castanopsis eyrei</i>	Tree	251	920	81	24	0.032	–	Microsatellites	Shi et al. (2011)
t1.22 <i>Fagus orientalis</i>	Tree	600	1900	100	6	0.015–0.046	–	Isozymes and microsatellites	Shanjani et al. (2011)
t1.23 <i>Fagus sylvatica</i>	Tree	992	1640	12	3	0.0135–0.0156	0.015	AFLP	Jump et al. (2006)
t1.24 <i>Larix kaempferi</i>	Tree	1300	2700	108	6	0.009	0.0023	Microsatellites	Nishimura and Setoguchi (2011)
t1.25 <i>Pinus brutia</i>	Tree	275	1050	20,000	9	0.053	–	Isozymes	Kara et al. (1997)
t1.26 <i>Pinus canariensis</i>	Tree	1035	2135	375	6	0.013–0.019	0.001–0.006	Microsatellites	Navascués et al. (2008)
t1.27 <i>Pinus pallasiiana</i>	Tree	150	900	–	4	0.017	–	Isozymes	Korshikov and Mudrik (2006)



t1.29	<i>Podocarpus parlatorei</i>	Tree	1040	3100	90.000	18	0.104	-	Isozymes	Quiroga and Premoli (2007)
t1.30	<i>Quercus crispula</i>	Tree	800	1800	2800	19	0.013	0.000	Microsatellites	Ohsawa et al. (2007)
t1.31	<i>Quercus serrata</i>	Tree	140	1200	2800	15	0.013	0.000	Microsatellites	Ohsawa et al. (2008)

t1.32  $F_{ST}$  a measure of population differentiation due to genetic structure, *RAPD* random amplified polymorphic DNA, *AFLP* amplified fragment length polymorphism

231 among those at dissimilar altitudes, altitudinal adaptation might be promoted by the  
232 spread of favorable alleles (Byars et al. 2009). In contrast, a prevailing counter-  
233 gradient gene flow across altitude might limit adaptation because gene swapping  
234 would prevent the maintenance of locally adapted individuals (Matter et al. 2013).  
235 Despite the large amount of literature on spatial genetic variation in mountainous  
236 species, we know little about actual patterns of gene flow with respect to altitudinal  
237 gradients. Therefore, the question remains: Which is more common – isolation by  
238 elevation or counter-gradient gene flow across altitude?

#### 239 ***4.2.5 Differentiations in Adaptive Traits and Non-neutral*** 240 ***Genes Along Altitudinal Gradients***

241 Natural selective pressures along an altitudinal gradient can also affect genetic  
242 variation in mountain plants. Since the classic studies of Clausen and his colleagues  
243 (Clausen et al. 1940; Clausen 1951), who established field experimental approaches  
244 to detect genetic differentiation in phenotypic traits and used common garden plants  
245 and reciprocal transplantation experiments to detect local adaptation, substantial  
246 evidence for adaptive genetic variation in plants along altitudinal gradients has  
247 accumulated. Intraspecific adaptation across altitudinal gradients has been reported  
248 in various plants, from woody conifers (Conkle 1973; Kitzmiller 2005; Ishizuka  
249 and Goto 2012) to herbs and grasses (Galen et al. 1991; Byars et al. 2007; Gonzalo-  
250 Turpin and Hazard 2009; Kenta et al. 2011). In addition, Gonzalo-Turpin and  
251 Hazard (2009) combined a field experimental study with a molecular approach to  
252 show that local adaptation can occur along altitude even in the presence of gene  
253 flow as estimated by neutral markers. In general, behavioral trends of neutral and  
254 adaptive genes differ both within and among populations because natural selection  
255 can contribute in various ways to heterogeneous genomic divergence. Genome  
256 scans have shown that usually only a small proportion of scored loci, generally  
257 5–10 %, consists of outliers that might be linked to genes implicated in the adaptive  
258 divergence (reviewed by Nosil et al. 2009), and some empirical studies have  
259 reported that only a few loci show non-neutral behavior associated with altitude  
260 that might drive natural selection (Jump et al. 2006; Shi et al. 2011; Manel  
261 et al. 2012). These studies observed very strong allele frequency differences  
262 along altitudinal gradients. However, very little is known about the locations and  
263 functions of specific genes involved in altitudinal adaptation (a notable exception is  
264 the albumin gene in deer mice; Stortz and Nachman 2003). The outlier genes  
265 mentioned above are appropriate targets for future investigations of linked functional  
266 genes, which can now be carried out by using high-throughput sequencing  
267 technology.

### 4.3 Case Studies: Altitudinal Trait Differentiation in Three Plant Species 268 269

#### 4.3.1 *Altitudinal Floral Adaptation to Local Pollinator Assemblages* 270 271

Ever since the seminal work of Clausen et al. (1940) in the Rocky Mountains, plants have been known to be physiologically and ecologically differentiated along altitudinal gradients (Byars et al. 2007; Angert 2009; Montesinos-Navarro et al. 2011). Typically, altitudinal changes in abiotic environments (e.g., meteorological changes) often cause the decrease in overall plant size with increasing elevation (Körner 2003; Hautier et al. 2009), but altitudinal changes in biotic interactions can also influence plant traits (Dohzono and Suzuki 2010). Studies exploring plant trait variations caused by such biotic interactions are scarce, however, though there are some exceptions (Galen 1985, 1989; Malo and Baonza 2002; Dohzono and Suzuki 2010; Sun et al. 2014). Because the modification of floral traits can easily lead to reproductive isolation and genetic differentiation in plants, studies of floral trait variations along altitude are particularly important for gaining understanding of altitudinal plant differentiation.

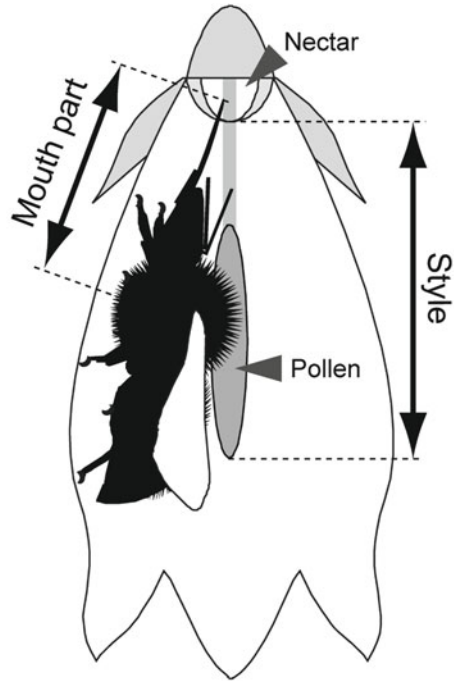
In angiosperms, selective pressure imposed by pollinators has played an important role in floral evolution (Stebbins 1970; Harder and Johnson 2009). Therefore, if pollinator assemblages differ among populations, local adaptations of floral traits may also be observed. In fact, floral traits have been shown to change geographically in relation to differences in pollinator assemblages (Galen 1996; Gómez et al. 2009; Nattero et al. 2010; Hattori et al. 2015; see Herrera et al. 2006 for a review), and this ecological differentiation of floral traits can potentially lead to genetic divergence among populations that become isolated as a result of specialization to specific pollinators (Nosil 2012).

Bumblebees are the dominant pollinators in the sub-alpine zone of Japanese mountains, and their species composition is known to change along altitude (Tomono and Sota 1997). Because a small bumblebee, *Bombus beaticola*, is strongly dominant above 1700 m a.s.l. in central Japan, bumblebee-pollinated plants at high altitude should have relatively smaller flowers than those growing at lower elevations, which are pollinated by larger bumblebee species. In the following sections, we describe two bumblebee-pollinated herbaceous species that show changes in floral size along an altitudinal gradient: *Campanula punctata* var. *hondoensis* and *Prunella vulgaris*.

##### 4.3.1.1 *Campanula punctata* 303

*Campanula punctata* var. *hondoensis* (Campanulaceae) grows across a wide altitudinal range (0–2300 m a.s.l.) in central Japan. Nagano et al. (2014) studied altitudinal changes in floral size and pollinator assemblages of 12 populations

**Fig. 4.1** Male-phase *Campanula punctata* flower and a visiting bumblebee. In this phase, the pollen grains adhere to the lateral surface of the style. Several days later, in the female phase, the tip of the style unfolds and the stigmatic lobes appear. The match between floral style length and pollinator mouthpart length is important for plant male fitness (i.e., removal of pollen during a bee's visit). See text for details (After Nagano et al. 2014)



307 situated at elevations from 744 to 2269 m a.s.l. in three mountain ranges and  
 308 examined the affect of the flower–pollinator size match on plant fitness.

309 In *Campanula* flowers (Fig. 4.1), pollen grains are not transferred directly from  
 310 the anthers to the pollinator; rather, pollen grains are shed onto the style while it is

still tightly surrounded by the anthers in the young bud. By the time the flower opens, the stamens have shriveled and the pollen adhering to the style is ready for transfer to visiting pollinators as they crawl into the corolla (male phase; Fig. 4.1). The stigma does not become receptive (female phase) until about 2 days after the flower opens.

The size match between flowers and bumblebees affects the male fitness of the plants but not their female fitness. Among different size-match indices investigated, only the ratio of the pollinator's mouthpart length (PL) to the floral style length (SL) (PL:SL; see Fig. 4.1) affected plant male fitness (defined as pollen removal from the flower style onto bees). While the bee is collecting nectar, pollen grains stick to the dorsal surface of its thorax. Thus, the PL:SL ratio determines the position of the bee's thorax on the style while the bee is collecting nectar. Because most grains adhere to the middle part of the style (about one-third of the distance from the tip), the optimal PL:SL should be around 2:3. Nagano and his colleagues in fact found that when the PL:SL ratio is larger or smaller than this value, pollen removal by the bumblebees is decreased. These results suggest that a large floral size should be selected for in locations (at altitudes) where larger bumblebee species are dominant.

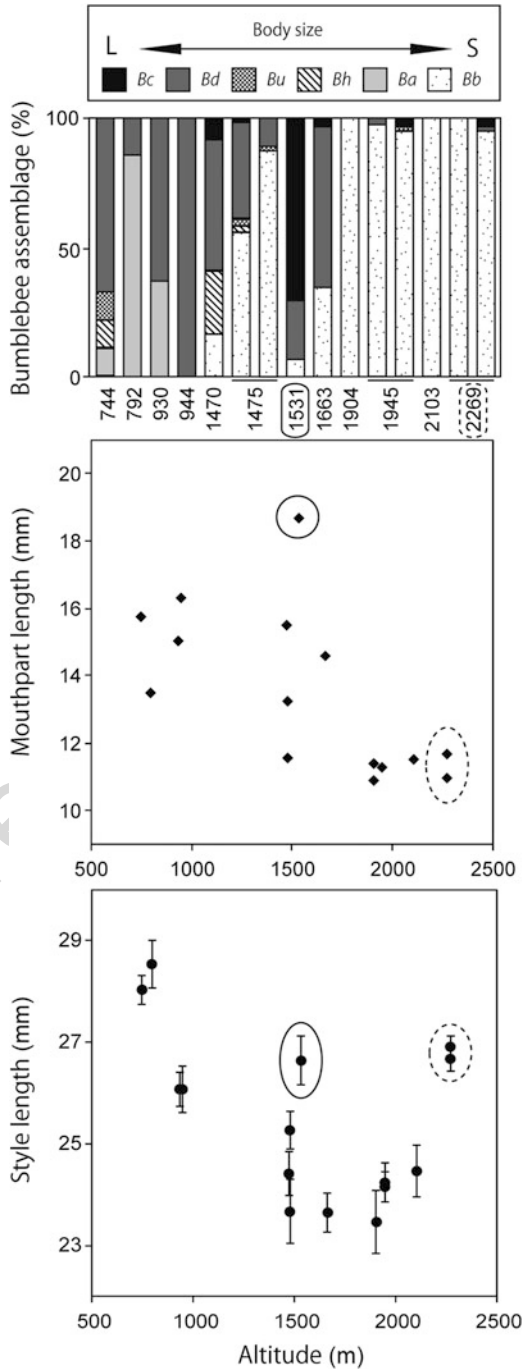
Nagano et al. (2014) also investigated the visitation frequency of bumblebee species to *C. punctata* flowers in the peak flowering season at sites at different elevations (Fig. 4.2, top) and calculated the average pollinator mouthpart length at each site from the relative abundance and average mouthpart length of each bumblebee species (Fig. 4.2, middle). They then examined whether bee size corresponded to floral size at different geographic locations (altitudes).

At high-altitude sites, where the small bumble bee *B. beaticola* was dominant (Fig. 4.2, top), the average pollinator mouthpart length was relatively short (Fig. 4.2, middle) and the style length of *C. punctata* also tended to be short (Fig. 4.2, bottom). However, bee and floral size did not simply decrease with elevation; rather, they changed in parallel although there is an exceptional site (circled by a dotted line in Fig. 4.2). Thus, floral size was large at a middle elevation site where the largest bumblebee species, *B. consobrinus*, was dominant (circled by a solid line in Fig. 4.2). By using generalized linear mixed models, Nagano et al. (2014) were able to show that the floral style length significantly correlated only with pollinator mouthpart length ( $P < 0.05$ ); it did not correlate with altitude ( $P = 0.40$ ) or with the number of flowers per plant (an index of plant size,  $P = 0.38$ ).

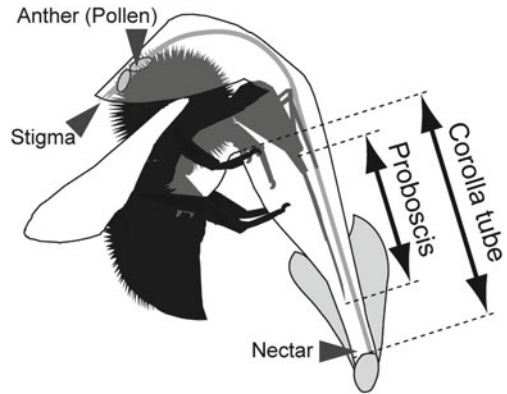
#### 4.3.1.2 *Prunella vulgaris*

*Prunella vulgaris* (Labiatae) is a perennial plant that grows in sunny mountain meadows of the temperate zone throughout the Northern Hemisphere. In central Japan, it blooms from June to August and is pollinated by bumblebees (Fig. 4.3). Kuriya et al. (2015) investigated geographical variations in the floral size of *P. vulgaris* and variations in the pollinator assemblage among seven *P. vulgaris* populations in the northern Japanese Alps, central Japan (1150–2050 m a.s.l.).

**Fig. 4.2** *Top*: Altitudinal change in assemblages of *C. punctata* pollinators (bumblebee). *Bb* *B. beaticola* workers and males, *Ba* *B. ardens* males, *Bh* *B. honshuensis* workers, *Bu* *B. ussuriensis* workers, *Bd* *B. diversus* workers, *Bc* *B. consobrinus* workers. In the figure key, mouthpart length is used as the index of bumble bee body size. The altitude of each population (m a.s.l.) is also indicated. Three populations were surveyed in 2 years. The largest bumblebee, *B. consobrinus* (worker), was the main visitor to the population encircled by the solid line. In the populations encircled by the dashed line, there was a mismatch between floral size and bumblebee size. *Middle*: Altitudinal variation of the average mouthpart length (see Fig. 4.1) of the pollinators of the 12 populations. Each diamond represents a population year. *Bottom*: Altitudinal variation (mean  $\pm$  SE) of floral style length (see Fig. 4.1) (After Nagano et al. 2014)



**Fig. 4.3** A *Prunella vulgaris* flower and a visiting bumblebee. The anthers and stigma of *P. vulgaris* are at the top of the corolla tube (After Kuriya et al. 2015)



The flowers of *P. vulgaris* are tubular with an upper hooded lip and a lower fringed lip, and the anthers and stigma lie along the top lip (Fig. 4.3). When a bumblebee visits a flower, the top of its head will touch the anthers and stigma if its proboscis is similar in length to the tubular corolla (Fig. 4.3, bottom). If a smaller bumblebee visits the same flower, however, it must crawl into the corolla to reach the nectar, and the dorsal side of its thorax will touch the anthers and stigma (Fig. 4.3, top). In either case, the pollen seems to be successfully transferred. Therefore, the bee–flower size match would not appear to be as important for plant fitness as it is in the case of *C. punctata*. Nevertheless, Kuriya et al. (2015) found that pollen removal by bees (an estimate of male fitness) and pollen deposition from a bee onto the stigma (female fitness) were both strongly affected by the bee–flower size match ( $P < 0.001$ , generalized linear model). Plant fitness was maximized when the length of the corolla tube was a little bit shorter than the

366 length of the bee's proboscis, that is, when the bee's thorax could just touch the  
367 anthers and stigma.

368 Kuriya and her colleagues also found that the primary visitor to high-altitude  
369 *P. vulgaris* populations was the small bumblebee *B. beaticola*, whereas the dom-  
370 inant pollinators at low-altitude sites were the medium-to-large bumblebees  
371 *B. diversus* and *B. ussuriensis* (Fig. 4.4, top). Overall, the proboscis length of the  
372 bees decreased along the altitudinal gradient (Fig. 4.4, middle), as was also the case  
373 with *C. punctata* pollinators (Fig. 4.2, middle).

374 Just as in *C. punctata* the style length tended to be shorter at higher elevations  
375 (Fig. 4.2, bottom), in *P. vulgaris* the corolla tube tended to be shorter at higher  
376 elevations (Fig. 4.4, bottom). One flower population at high elevation, however, had  
377 long corolla tubes (Fig. 4.4, bottom), and the large bumblebee *B. consobrinus* was  
378 the dominant visitor to this *P. vulgaris* population (Fig. 4.4, top). Kuriya and her  
379 colleagues showed by a generalized linear model that the corolla tube length of each  
380 population was significantly correlated only with bee proboscis length ( $P < 0.001$ )  
381 and not with inflorescence length ( $P = 0.74$ ) or altitude ( $P = 0.44$ ).

### 382 4.3.1.3 Synthesis

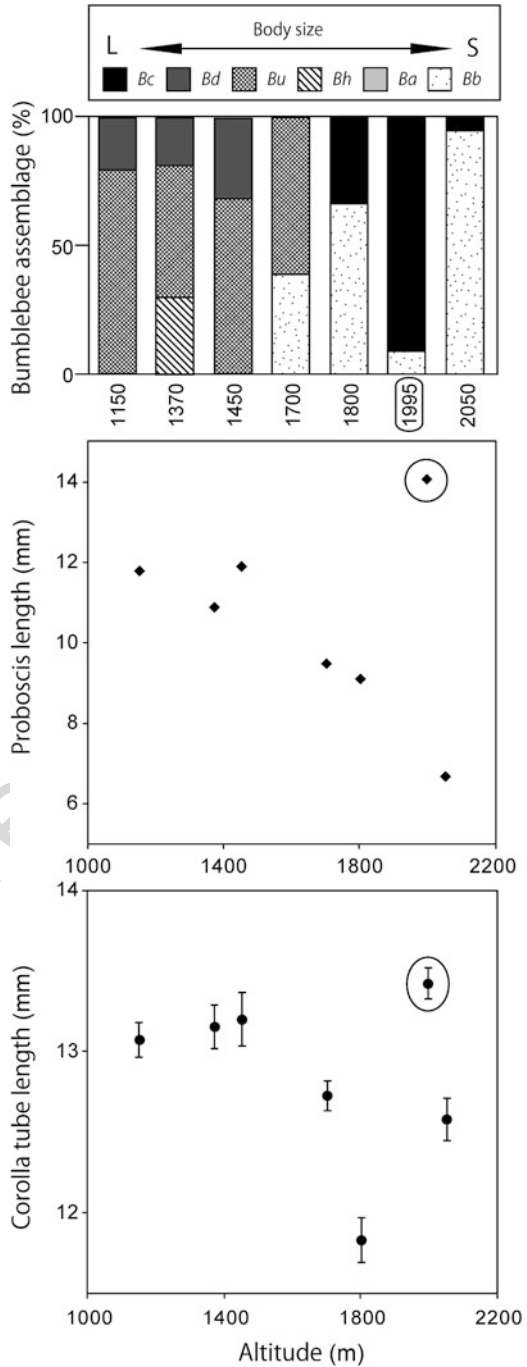
383 The local floral size of both *C. punctata* and *P. vulgaris* correlated only with the  
384 local pollinator size. At least two possible mechanisms might account for this  
385 correlation: (1) an adaptive floral size may be selected for by the local bee size  
386 (bees-came-first hypothesis), or (2) local floral size may vary because of unknown  
387 (climatic or edaphic) factors and each bumblebee species preferentially visits the  
388 right-sized flowers (flowers-came-first hypothesis).

389 If the flowers-came-first hypothesis is correct, then the larger bees such as  
390 *B. diversus* should prefer larger *C. punctata* (*P. vulgaris*) flowers and smaller  
391 bees such as *B. beaticola* should prefer smaller flowers in the same *C. punctata*  
392 (*P. vulgaris*) population. However, Nagano et al. (2014) detected no floral size  
393 preference of *B. diversus* or *B. beaticola* in populations of *C. punctata* visited by the  
394 both bumblebee species. This result suggests that the floral size of *C. punctata* does  
395 not influence the flower visitor assemblage of *C. punctata*. In addition, as described  
396 in Sects 4.3.1.1 and 4.3.1.2, in each population of *C. punctata* and *P. vulgaris*, floral  
397 size correlated only with the pollinator mouthpart length and not with altitude,  
398 number of flowers per plant, or inflorescence length. This finding suggests that  
399 floral size is not influenced by climatic or edaphic factors but by bee size. Further,  
400 plant fitness was maximized when floral size was matched to bee size, which  
401 suggests adaptive local floral evolution. These results strongly suggest that varia-  
402 tion in local bee assemblages along altitude is exerting selective pressure on the  
403 floral size of *C. punctata* and *P. vulgaris*.

404 The altitudinal variation of bumblebee assemblages (Figs. 4.2 and 4.4, top)  
405 found in these two case studies generally reflects their altitudinal distribution in  
406 central Japan (Tomono and Sota 1997), whereas variation detected within the same



**Fig. 4.4** *Top:* Altitudinal change in assemblages of *P. vulgaris* pollinators. The abbreviations of the bumblebee species are the same as in Fig. 4.2. The largest bumblebee, *B. consobrinus* (worker), was the main visitor to the population encircled by a solid line. *Middle:* Altitudinal variations of the average proboscis length (see Fig. 4.3) of the pollinators of the seven populations. Each diamond represents a population year. *Bottom:* Altitudinal variation (mean  $\pm$  SE) of floral corolla length (see Fig. 4.3) (After Kuriya et al. 2015)



407 altitudinal range (Figs. 4.2 and 4.4, top) may reflect the influence of bumblebee nest  
 408 location, which depends on the distribution of floral resources during the nest-  
 409 initiating period of bumblebee queens (Suzuki et al. 2007, 2009). The correlation of  
 410 pollinator (bumblebee) assemblages and floral traits of these two plant species  
 411 along altitude suggests that the floral traits of other bumblebee-pollinated plants  
 412 might also be influenced by local bumblebee assemblages along altitude. Future  
 413 studies, therefore, should focus not on a single plant species but on multiple  
 414 sympatric species to elucidate how local pollinator assemblages influence the  
 415 formation of concerted floral trait evolution along altitude (Hattori et al. 2014,  
 416 2015).

### 417 4.3.2 *Cimicifuga simplex*: Ecological and Genetic 418 Differentiation Along Altitude

419 *Cimicifuga simplex* (Ranunculaceae) is a perennial herb widely distributed in East  
 420 and Northeast Asia. In Japan, it grows from sea level to over 3000 m (Pellmyr  
 421 1986). Pellmyr (1986) grouped Japanese *C. simplex* into three pollination morphs  
 422 based on their pollinator guilds and other traits. Around Nikko in central Honshu,  
 423 morph I occurs at altitudes over 1300 m and typically reaches 1.5–2.5 m in height  
 424 (Pellmyr 1986). Morph II occurs at or below 950 m and is as tall as morph I. Morph  
 425 III, which grows in very dark, moist habitats within and below the altitudinal range  
 426 of morph II, is less than 1.0 m tall. Morphs I and III are pollinated by bumblebees  
 427 (Hymenoptera, Apidae), whereas morph II is frequently visited by fritillary butter-  
 428 flies (Fig. 4.5; Lepidoptera, Nymphalidae). Morph II is distinguishable from  
 429 morphs I and III by its floral fragrance (Pellmyr 1986; Groth et al. 1987), whereas  
 430 morph III can be distinguished from the other two morphs by its lower plant height  
 431 and later flowering season.

432 Working independently of Pellmyr (1986), Yamaji et al. (2005) divided  
 433 *C. simplex* into seven genotypes based on polymorphism of ITS sequences of  
 434 nuclear ribosomal DNA. Whether any of these genotypes correspond to the polli-  
 435 nation morphs of Pellmyr (1986) has not yet been investigated. However, because  
 436 the three pollination morphs of Pellmyr (1986) are distributed parapatrically or  
 437 allopatrically along altitude and have clearly different morphological and

t2.1 **Table 4.2** Floral characters of the three pollination morphs of *Cimicifuga simplex* (after Kuzume  
 and Itino 2013)

t2.2	Pollination morph	Floral fragrance	Flowering period	Major flower visitors	Altitudinal distribution (m, a.s.l)
t2.3	I	Not sweet	August–September	Bumblebees	1500–2400
t2.4	II	Sweet	September	Butterflies	1100–1500
t2.5	III	Not sweet	September–October	Flies and syrphids	800–1000

**Fig. 4.5** Fritillary butterflies (Lepidoptera, Nymphalidae) visiting a *Cimicifuga simplex* plant (pollination morph II)



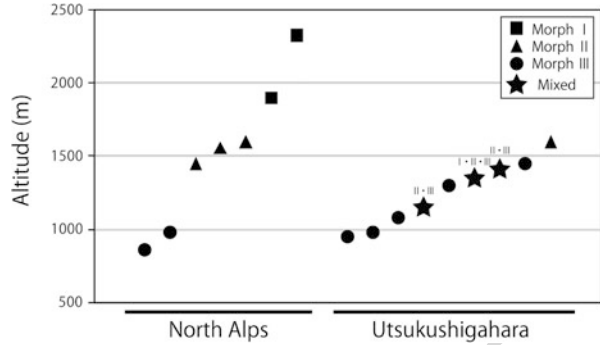
ecological traits, it is reasonable to hypothesize that they are genetically differentiated as well. 438 439

Accordingly, Kuzume and Itino (2013) surveyed the altitudinal distribution of the three pollination morphs of *C. simplex* in 16 populations along an altitudinal gradient (800–2400 m) in the northern Japanese Alps and in the Utsukushigahara highlands of central Japan, determined the genotypes and compared the morphological traits of the three morphs. In these two regions, which are about 50 km apart, the altitudinal distributions of the pollination morphs were basically the same, and they also were in accord with Pellmyr’s (1986) description (Table 4.2). Morphs I, II, and III were found at high, medium, and low elevations, respectively, although the different morphs sometimes coexisted in the same population in the Utsukushigahara highlands (Fig. 4.6). In these mixed populations, individuals with intermediate morphological characteristics suggesting hybridization were not observed, indicating that the morphs are probably reproductively isolated. 440 441 442 443 444 445 446 447 448 449 450 451

Kuzume and Itino (2013) determined the ITS sequences of the three pollination morphs and found that, without exception, morphs I, II, and III corresponded to three of the seven ITS genotypes described by Yamaji et al. (2005); they did not detect the other four genotypes. In accordance with the genetic differentiation of the pollination morphs, they also found that the degree of pubescence of the leaf margin was a diagnostic external character that could be used to discriminate pollination morph II from morphs I and III. 452 453 454 455 456 457 458

Thus, in central Japan, the *C. simplex* populations are composed of at least three genetically differentiated ecotypes, which are distributed parapatrically or allopatrically along an altitudinal gradient. 459 460 461

**Fig. 4.6** Altitudinal distribution of the three pollination morphs of *Cimicifuga simplex* in the northern Japanese Alps and the Utsukushigahara highlands of central Japan



#### 462 **4.4 Concluding Remarks: Conserving Intraspecific** 463 **Variation Along Altitudes**

464 Steep altitudinal environmental changes cause remarkable clinal changes in species  
465 composition and species diversity. Although possible effects of climate warming on  
466 diversity at the species or community level along an altitudinal (or latitudinal)  
467 gradient have attracted considerable attention recently (Sanders and Rahbek 2012),  
468 those on intraspecific diversity have attracted less attention (Pauls et al. 2013).  
469 Studies of intraspecific diversity have mainly investigated altitudinal patterns of  
470 genetic diversity, and few examples of neutral genetic differentiation along altitude  
471 have been identified (Table 4.1). Rather, the prevailing counter-gradient gene flow  
472 appears to hinder altitudinal differentiation of neutral genes.

473 In contrast, a substantial number of studies have shown that, regardless of the  
474 frequency of gene flow, altitudinal differences in selective pressure cause adaptive  
475 differentiation of physiological, morphological, or life history traits along altitude  
476 (Kenta et al. 2011; Ishizuka and Goto 2012 and references therein). Our survey of  
477 the literature suggests that intraspecific differentiation of neutral genes is relatively  
478 uncommon whereas that of adaptive traits is more common. In either case, for  
479 effective conservation management of mountain plants, it is important to elucidate  
480 the degree and intensity of intraspecific differentiation along altitude.

481 The three case studies on genetic (*C. simplex*) and phenotypic (*C. punctata* and  
482 *P. vulgaris*) differentiations along altitudes suggest that recognized taxonomic  
483 species are often composed of several different evolutionary or ecological units,  
484 thus providing new insight into our understanding of biodiversity in mountain  
485 ecosystems. From the viewpoint of conservation, the intraspecific diversification  
486 documented here broadens the scope of our understanding of the threat posed by  
487 climate warming to mountain biodiversity. Previous investigations focused on the  
488 potential loss of species- and community-level diversity (Gottfried et al. 2012; Pauli  
489 et al. 2012), whereas here we have shown that climate warming may lead to a loss of  
490 intraspecific diversity. Because the distribution range of intraspecifically differenti-  
491 ated types (e.g., the high-altitude morph of *C. simplex*) along altitude is typically  
492 narrower than that of recognized taxonomic species, populations are more likely to

be extirpated, or, in the worst case, the type may become extinct in the face of climate warming and the resultant range shift. In addition, intraspecific types are more likely to lose genetic variation because they are relatively less abundant than the species as a whole; thus, their ability to adapt is limited.

The altitudinal phenotypic variations in *C. punctata* and *P. vulgaris* suggest that inter-altitudinal gene flow occurs and sharply defined ecotypes are thus absent. However, the observed ecological variation along altitude in these species still has important implications for their conservation. For example, if the small bumblebee, *B. beaticola*, were to become extinct because of climate warming, the adaptive floral variation (small-sized flower) of the high-altitude plant variants that are specially adapted to this bee species would be lost, and the type would become extirpated.

To ensure the conservation of mountain plants in the face of future climate warming, it is first necessary to clarify to what extent such plants are genetically and ecologically differentiated along altitude. Then, further studies should identify intraspecific types or variants and their predicted range shift (Chen et al. 2011), adaptive potential (Till-Bottraud and Gaudeul 2002; Byars et al. 2007; Hoffmann and Sgrò 2011), and plastic phenotypic changes in response to climate change (Canale and Henry 2010). The results of these studies could then be used to develop a framework for the conservation of intraspecifically and altitudinally variable mountain plant species.

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