

**PATTERNS OF GENETIC DIVERSITY IN COLONIZING PLANT SPECIES: *NASSAUVIA LAGASCAE* VAR. *LANATA* (ASTERACEAE: MUTISIEAE) ON VOLCÁN LONQUIMAY, CHILE<sup>1</sup>**

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The effect of colonization on the distribution of genetic diversity within and among populations in relation to species characteristics remains an open empirical question. The objective of this study was to contrast genetic diversity within and among established and colonizing populations of *Nassauvia lagascae* var. *lanata* on Volcán Lonquimay (Araucanía Region, Chile), which erupted on 25 December 1988, and relate genetic diversity to biological characteristics of the populations. We analyzed a total of 240 individuals from 15 populations distributed along the Andes Cordillera using AFLP and obtained a total of 307 AFLP bands, of which 97.7% are polymorphic. Values of population differentiation ( $F_{ST}$ ) did not differ significantly among established and colonizing populations, but colonizing populations did have reduced levels of genetic divergence (as indicated by private and rare bands) and genetic variation (e.g., Shannon index). We conclude that a founder effect through limited numbers of founding propagules derived from nearby source populations has not yet been compensated for by subsequent population growth and migration. Low rates of secondary dispersal via running water, kin-structure within populations, and slow population growth seem to contribute to the slow recovery of genetic diversity.

**Key words:** AFLP; Andes; Asteraceae; colonization; Compositae; dispersal ability; Mutisieae; *Nassauvia*; population genetic parameters; volcanoes.

Disturbances in the landscape created by volcanic activity offer an excellent opportunity to study the effect of such disturbances on the genetic structure and variability within and among populations. Extinction and recolonization after local disturbances could result in sampling from the available gene pool (founder effect) or in additional gene flow. The relative contributions of these opposing forces in different plant systems and their consequences for the genetic composition of the species as a whole remain open empirical questions.

From a population genetic standpoint, the founder effect impacts allele frequencies and genetic diversity in colonizing populations, whereby immigrant seeds carry only a small

sample of alleles from the source population (Slatkin, 1977; Pannell and Charlesworth, 1999; Silvertown and Charlesworth, 2001). The founder event is associated with a decline in genetic diversity, because it is less likely that rare alleles are included in the colonizing individuals, thus favoring the most common alleles. It was early recognized that reduction in average heterozygosity depends on both the size of the bottleneck (or strength of the founder effect) and the rate of population growth (Nei et al., 1975). If population size increases rapidly after going through a bottleneck (or founder event), the reduction in average heterozygosity is rather small. Loss in the average number of alleles per locus, however, is profoundly affected by bottleneck size (Nei et al., 1975). Slatkin (1977) emphasizes the importance of the mode of establishment of new populations for genetic variability in colonizing populations. According to him, colonization after local extinction has two consequences. The first is an additional sampling process similar to genetic drift resulting from the sampling of the colonizing individuals from their source populations (founder effect). The second is an additional component to gene flow between the local populations, because the colonizing individuals originate from one or more of the local populations. The direction and the magnitude of the effect of colonization after local extinction are then dependent on the relative contributions of the two opposing forces (genetic drift vs. gene flow). Wade and McCauley (1988) refine Slatkin's (1977) models and relate the effect of the extinction/colonization process on genetic variability to the source of propagules (from one or several local

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populations) and the relative number of propagules colonizing vacant habitats (compared to the number of migrants between extant populations). Whitlock and McCauley (1990) add the importance of kin structure and inbreeding within colonizing populations as critical factors affecting genetic variability after extinction and colonization, whereby kin structure and inbreeding lead to increased population differentiation.

With time, migration would reduce the differentiation between populations caused by colonization (Pannell and Dorken, 2006). Concomitantly, the immigration process after colonization determines the speed at which the population can recover the genetic variation lost during the founder effect (Ingvarsson, 1997). In summary, results from population genetic theory suggest that a variety of factors impact allele frequencies and genetic diversity in colonizing populations. These include the relative number of founding propagules in comparison to migrants among extant populations; the probability of common origin of the founding propagules; kin structure and inbreeding within the colonizing populations; and the rate of population growth and immigration after colonization.

Colonization of landscape gaps created as a result of volcanic activity offers an excellent opportunity for studying the genetic diversity and structure of colonizing and surviving populations. The number of studies on continental environments, however, is small, and most of them are concerned with the settlement of Mount St. Helens, USA (del Moral and Wood, 1993; del Moral, 1998; del Moral and Eckert, 2005; del Moral and Lacher, 2005; Yang et al., 2008). In this context, it is profitable to examine Volcán Lonquimay (located in the southern Andes of Chile at ~38° S), which experienced a major eruption on 25 December 1988, causing the formation of a new side cone, Navidad. The emission column during the activity of the volcano reached 9000 m, with a total volume of lava emitted of around 180 000 000 m<sup>3</sup>, which mainly covered old lava deposits. Acid rain, falling ash (to the southeast), and lava flow resulted in the destruction of the surrounding vegetation. Due to the intensity of the eruption, no diaspores are believed to have survived in the area covered with ash. In the years following the eruption, specialized colonizers arrived, including *Chaetanthera villosa*, *Hypochaeris tenuifolia*, *Nassauvia argentea*, and *N. lagascae* var. *lanata* (all Asteraceae), *Loasa nana* (Loasaceae), and *Pozoa volcanica* (Apiaceae).

This study seeks to determine the levels of genetic diversity in established and colonizing populations of *Nassauvia lagascae* var. *lanata* located in areas around the Navidad cone of Volcán Lonquimay and also to infer the relationships among established and colonizing populations in the Lonquimay and surrounding area. We selected the AFLP (Vos et al., 1995) fingerprinting technique because of its high efficacy to reveal patterns of genetic diversity in natural populations (e.g., Gaudeul et al., 2000; Nybom, 2004; Andrade et al., 2009). Furthermore, we relate the levels of genetic diversity in established vs. colonizing populations with population size as well as environmental and biological characteristics of the populations (e.g., vegetation coverage, attributes of vegetative growth and reproduction). The results are discussed in comparison with previous results from *Hypochaeris tenuifolia* (Asteraceae) from the same study area (Tremetsberger et al., 2003). In contrast to *Hypochaeris*, *Nassauvia* has low dispersal ability (Castor, 2002). We therefore expect to find evidence for a founder effect in colonizing populations of *Nassauvia lagascae* var. *lanata*.

## MATERIALS AND METHODS

**The species**—*Nassauvia lagascae* (D. Don) F. Meigen var. *lanata* (Phil.) Skottsb. (Kongl. Svenska Vetensk. Acad. Handl. 56(5): 329. 1916) is a perennial, cushion-forming herb, with ascending or decumbent stems, a few centimeters high, and densely covered with leaves up to the apex. Leaves are imbricate, oblanceolate-spatulate to obovate-espulate, recurvate, and densely woolly on the lower side. Numerous capitula are arranged in very dense globose spikes at the tips of the branches. The involucre is cylindrical with woolly phyllaries. Flowers are white and smell subtly sweetish. Achenes (cypselas) are glabrous, with a pappus consisting of numerous linear plumose bristles (Cabrera, 1982), which detaches easily from the rest of the fruit. No experiments have been carried out to determine the breeding system of *Nassauvia* species, but the white, fragrant flowers suggest an outcrossing mode. The variety grows in the Andes from the Maule Region to the Araucanía Region in Chile and from the south of the Mendoza Province to the Santa Cruz Province in Argentina (~35–52°S) (Cabrera, 1982).

**Sampling**—In the Lonquimay and surrounding area (Araucanía Region, Chile), *N. lagascae* var. *lanata* grows in an altitude of ~1500–2200 m a.s.l. Thus, it has an island-like distribution on the volcanoes and mountain tops. Because the focus of this study is to compare genetic composition of established and colonizing populations, we put the emphasis of our sampling in the Lonquimay and surrounding area. We sampled six established populations (three in the immediate vicinity of Volcán Lonquimay and three in the surrounding area [Sierra Nevada, Llaima, and Pino Hachado]), seven colonizing populations (growing on the ash fields of the December 1988 eruption of the Navidad cone), and one population growing on ash from an older eruption of Volcán Lonquimay (Table 1, Fig. 1). Two populations further north (Chillán, Biobío Region, Chile, and Copahue, Neuquén Province, Argentina) and one population farther south (Villarrica, Araucanía Region, Chile) were also sampled. Additional potential habitats of *N. lagascae* var. *lanata* in the farther adjacencies of Volcán Lonquimay, from which we do not have material, include Volcán Callaqui and the Nevados de Sollipulli. Leaves of 16 individuals per population were collected on silica gel. Individuals were chosen randomly throughout the area occupied by the populations. Vouchers of each population sampled are on deposit in the herbarium WU.

Established populations in the Lonquimay and surrounding area (populations [pops.] 3–7) grow on volcanic lava and ash as well as nonvolcanic, siliceous

TABLE 1. Collection data of populations of *Nassauvia lagascae* var. *lanata* in Chile used for the AFLP study. Vouchers are deposited at WU. Populations 6A and 13A have not been subjected to AFLP analysis.

Region	Population	Collection number	Latitude (S)	Longitude (W)	Elevation (m a.s.l.)
North	1: Chillán	KT et al., 1018	36°54'08"	71°23'46"	2190
	2: Copahue	KT et al., 1034	37°49'53"	71°06'44"	2120
Volcán Lonquimay and surrounding area					
Established populations					
	3: Cerros de Lanco	KT et al., 1066	38°20'54"	71°25'47"	1835
	4: Tolhuaca	KT et al., 1087	38°21'02"	71°36'13"	1830
	5: Colorado	KT et al., 17	38°24'40"	71°34'34"	1900
	6: Sierra Nevada	KT et al., 64	38°36'54"	71°35'45"	1940
	6A: Llaima	KT et al., 110	38°41'26"	71°46'27"	1960
	7: Pino Hachado	KT et al., 1041	38°39'30"	70°53'50"	1900
Colonizing populations (since eruption of cone Navidad, December 1988)					
	8: Lonquimay	KT et al., 1050	38°21'50"	71°31'16"	2000
	9: Lonquimay	KT et al., 1049	38°21'50"	71°31'37"	1910
	10: Lonquimay	KT et al., 1071	38°22'05"	71°31'48"	1950
	11: Lonquimay	KT et al., 1067	38°22'12"	71°32'18"	1950
	12: Lonquimay	KT et al., 97	38°22'49"	71°33'13"	1930
	13: Lonquimay	KT et al., 1047	38°23'17"	71°32'21"	1975
	13A: Lonquimay	KT et al., 32	38°23'09"	71°32'22"	1960
Population growing on ash from an older eruption of Volcán Lonquimay					
	14: Lonquimay	KT et al., 11	38°24'32"	71°34'06"	1670
South	15: Villarrica	KT et al., 1079	39°23'57"	71°57'46"	1520

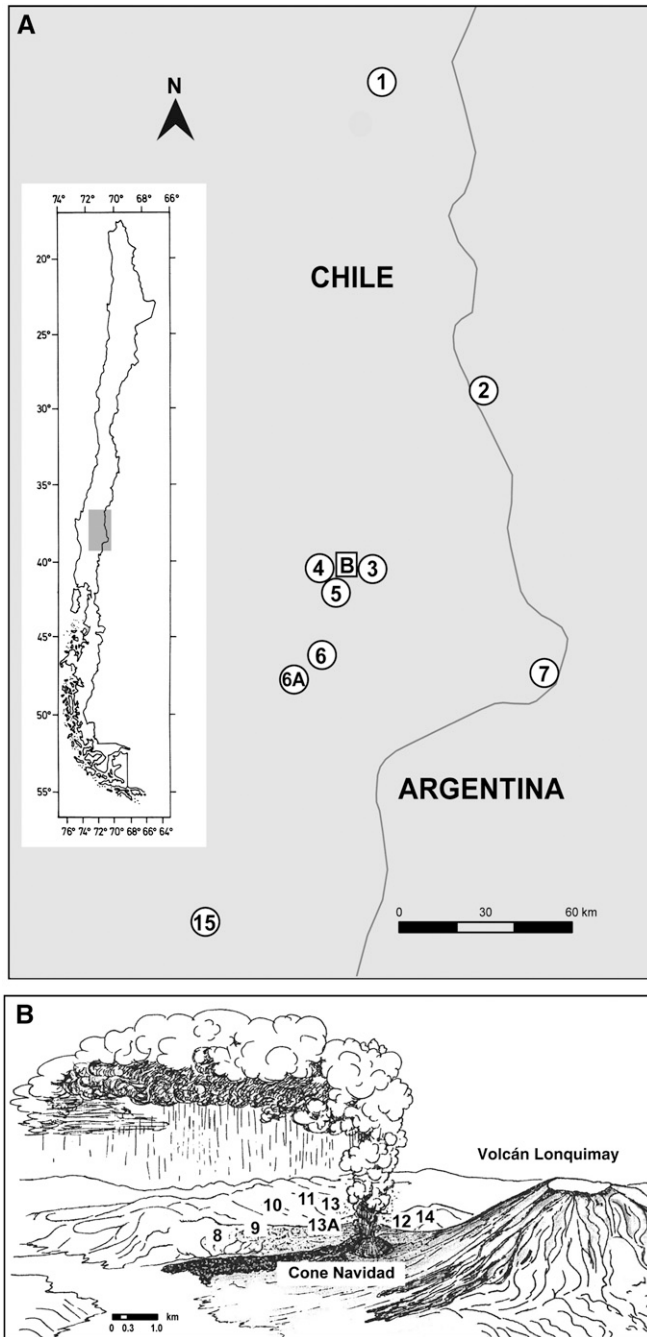


Fig. 1. Map of populations of *Nassauvia lagascae* var. *lanata* sampled in the Andes Cordillera. (A) Established populations (pops. 1–7 and 15). (B) The volcanic eruption site (modified from González-Ferrán [1994]) with colonizing populations (pops. 8–14).

debris and sand. The altoandine vegetation includes *Adesmia longipes* (Fabaceae), *Azorella* spp. (Apiaceae), *Cerastium arvense* (Caryophyllaceae), *Empetrum rubrum* (Empetraceae), *Ephedra andina* (Ephedraceae), *Gamocarpha alpina* (Calyceraceae), *Haplopappus* spp. (Asteraceae), *Loasa nana* (Loasaceae), *Mulinum spinosum* (Apiaceae), *Oreopolus glacialis* (Rubiaceae), *Oxalis adenophylla* (Oxalidaceae), *Poa* spp. (Poaceae), *Pozoa volcanica* (Apiaceae), and *Senecio* spp. (Asteraceae).

The colonizing populations (pops. 8–13A) grow on volcanic ash. The soil consists of a >15 cm thick layer of volcanic ash homogeneously mixed with very little organic material. In two populations (pops. 11 and 13A), a stony

brown soil is topped by 5 cm volcanic ash. The competitors include *Chaetanthra villosa* (Asteraceae), *Hypochaeris tenuifolia* (Asteraceae), *Nassauvia argentea* (Asteraceae), *Loasa nana*, *Oxalis adenophylla*, *Poa* spp., and *Pozoa volcanica*. Population 14 grows on ash from an older eruption of the volcano with *Euphorbia collina* (Euphorbiaceae), *Loasa nana*, *Phacelia secunda* (Hydrophyllaceae), and *Poa* spp.

The northern populations (pops. 1 and 2) were collected in stable scree and earth on volcanic and nonvolcanic substrate with typical altoandine vegetation; the competitors include *Acaena* spp. (Rosaceae), *Adesmia* spp., *Draba gilliesii* (Brassicaceae), *Gamocarpha alpina*, *Nassauvia revoluta*, *Olsynium junceum* (Iridaceae), *Pozoa coriacea*, and *Senecio* spp.

Finally, the southern population (pop. 15) grows on stable scree and earth, in volcanic lava and ash. The competitors include *Adesmia emarginata*, *Gaultheria phillyreifolia* (Ericaceae), *Nassauvia revoluta*, *Poa* spp., and *Senecio* spp.

**Population characteristics**—For each population, we estimated the following parameters to relate them to inferences of genetic diversity: total number of individuals, area occupied (m<sup>2</sup>), average diameter of plants (cm), average height of plants (cm), number of shoots per individual (median of 10 plants), proportion of reproductive individuals (with flowers or fruits), number of flowering shoots per reproductive individual (median of 10 plants), as well as coverage and height of the herb layer. In all populations, we searched for seedlings. The Mann–Whitney *U* test was used to estimate the significance of differences in population characteristics between established and colonizing populations in the Lonquimay and surrounding area using the program SPSS ver. 15.0 (SPSS, Chicago, Illinois, USA).

**AFLP fingerprinting**—We scored 240 individuals from 15 populations of *N. lagascae* var. *lanata* for three AFLP primer combinations (populations 6A and 13A have not been subjected to AFLP analysis). Genomic DNA was extracted from silica-gel-dried leaf material following the CTAB method (Doyle and Doyle, 1987) with minor modifications (Tremetsberger et al., 2003). The AFLP protocol followed Vos et al. (1995) with modifications as indicated in Tremetsberger et al. (2003). The selective primer combinations chosen following a primer-trial are *MseI*-CTAG/*EcoRI*-ACT (Fam), *MseI*-CACC/*EcoRI*-ACG (Hex), and *MseI*-CATA/*EcoRI*-ACC (Ned). The software Genographer ver. 1.6.0 (Benham, 2001) was used for scoring AFLP bands. Presence and absence of bands of 100–500 bp were scored in all individuals in a single file after normalizing on total signal. Criteria for selecting AFLP bands were visual clarity, straightforward interpretability, and similar fluorescence intensity across individuals. Cutoff levels were adjusted for each selected band, and automatic scores were visually checked and modified if necessary.

**Estimation of divergence of populations and within-population genetic variation**—The number of different AFLP phenotypes present in a population was counted with the program Arlequin ver. 3.1 (Excoffier et al., 2006). Divergence of populations was estimated via the occurrence of private bands, i.e., those bands confined to only one population, and rare bands. The number of private bands in each population was counted using the program FAMVD ver. 1.108 (Schlüter and Harris, 2006). The rarity index or DW (frequency-downweighted marker values) was first applied by Schönswetter and Tribsch (2005) for AFLP data, but is equivalent to range-downweighted values for species in historical biogeographical research (Crisp et al., 2001). The index was calculated with R-script AFLPdat (Ehrich, 2006; last modified 23 January 2008) in the program R ver. 2.6.0 (R Foundation for Statistical Computing; available at website <http://www.r-project.org/>). For each individual, each AFLP band was divided by the total number of occurrences of this band in the data set. These relative values were then added to the rarity index for this particular individual. Population values were estimated as the average of the individual values. The presence of private and rare bands is characteristic of populations with a long in situ history, most probably going back to the last glaciation (Schönswetter and Tribsch, 2005; Ehrich et al., 2008).

Within-population genetic variation was assessed for each population by the total number of AFLP bands, percentage of polymorphic bands (by dividing the number of polymorphic bands by the total number of bands in the dataset), and Shannon diversity index  $H_{Sh} = -\sum [p_i \times \ln(p_i)]$ , where  $p_i$  is the frequency of the  $i^{th}$  band in the respective population based on all AFLP bands recorded using the program FAMVD ver. 1.108 (Schlüter and Harris, 2006). The Pearson correlation was used to test correlation among different estimates of genetic variation using SPSS ver. 15.0 (SPSS). The Mann–Whitney *U* test was used to estimate the significance of differences of divergence of populations and within-population genetic variation between established and colonizing populations in the Lonquimay and surrounding area using SPSS.

**Estimation of population differentiation**—Genetic differentiation among local populations was assessed by analysis of molecular variance (AMOVA) using Arlequin ver. 3.1 (Excoffier et al., 2006), where total genetic diversity was partitioned into components among two hierarchical levels, among populations ( $F_{ST}$ ) and among individuals within populations. An alternative Bayesian approach (Holsinger et al., 2002) was used to obtain an independent estimate of  $F_{ST}$  in established and colonizing populations. This method allows estimation of  $F_{ST}$  from dominant markers without assuming Hardy–Weinberg proportions in populations. The original data matrix was imported into the program Hickory ver. 1.1 (Holsinger and Lewis, 2003–2007) and used for a full model,  $f = 0$  model,  $\theta = 0$  model, and  $f$ -free model run with default parameters (i.e., the hickory block omitted). The  $f$ -free model, which estimates  $\theta$  without estimating  $f$  (thus incorporating all the uncertainty in the prior of  $f$ ), is available for dominant marker data, because estimates of  $f$  derived from dominant marker data may be unreliable. The deviance information criterion (DIC; Spiegelhalter et al., 2002) was used to estimate how well a particular model fits the data and to choose between models.

**Population structure**—To examine the population structure of *Nassauvia lagascae* var. *lanata* we performed Bayesian clustering using the program BAPS ver. 5.1 (Corander et al., 2003, 2004; Corander and Martinen, 2006), which uses stochastic optimization to find the optimal partition. Simulations were run from  $K = 2$  to  $K = 16$  with five replicates for each number of clusters ( $K$ ). Admixture clustering based on results of mixture clustering was performed with the following settings: minimal size of clusters at five individuals, 100 iterations to estimate the admixture coefficients for the individuals, 200 simulated reference individuals from each population, and 20 iterations of each reference individual.

To construct a phenogram representing genetic distances among populations, population-pairwise  $F_{ST}$  values were generated using Arlequin ver. 3.1 (Excoffier et al., 2006). The  $F_{ST}$  values were used to construct a neighbor-joining (NJ) tree in the program PAUP\* ver. 4.0b10 (Swofford, 2002). Support for each node was tested with 500 bootstrap replicates of the NJ method in conjunction with Nei and Li's (1979) genetic distances on the original presence/absence matrix in PAUP\*.

## RESULTS

**AFLP**—The total number of AFLP bands found in all individuals and all populations is 307, of which 300 (97.7%) are polymorphic. The primer combination *MseI*-CTAG/*EcoRI*-ACT (Fam) yielded 104 bands in the range of 100–486 bp, *MseI*-CACAC/*EcoRI*-ACG (Hex) yielded 96 bands in the range of 104–474 bp, and *MseI*-CATA/*EcoRI*-ACC (Ned) yielded 107 bands in the range of 100–440 bp. All individuals have unique AFLP phenotypes.

**Divergence of populations and within-population genetic variation**—The number of private bands and the rarity index were used to estimate divergence of populations. In the Lonquimay and surrounding area, the established populations (pops. 3–7) have significantly higher values for these indices than the colonizing populations (pops. 8–13; Table 2, Fig. 2A). Population 14 on ash from an older eruption has a low value for the rarity index, similar to the colonizing populations. The northern populations (pops. 1 and 2) and the southern population (pop. 15) have comparably high values (similar to those found in the established populations of the Lonquimay and surrounding area).

The three estimates of genetic variation, total number of bands, percentage of polymorphic bands, and Shannon diversity, are all correlated. For example, the Pearson correlation between Shannon diversity and total number of bands is  $r = 0.967$  ( $N = 15$ ,  $P$  [2-tailed] = 0.000) and between this index (Shannon) and percentage of polymorphic bands  $r = 0.974$  ( $N = 15$ ,  $P$  [2-tailed] = 0.000). The estimates of genetic variation vary among populations (Table 2, Fig. 2B). In the Lonquimay and surrounding area, the established populations (pops. 3–7)

have on average higher values for all three estimates of genetic variation than the colonizing populations (pops. 8–13), although the differences are not significant. Population 14 on ash from an older eruption has low values for estimates of genetic variation, similar to the colonizing populations. The northern populations (pops. 1 and 2) have comparably low values, and the southern population (pop. 15) has intermediate values.

**Among-population genetic diversity and geographical structure**—Analysis of molecular variance (AMOVA) attributes 15.5% variance ( $df = 14$ ) among the 15 populations and 84.5% variance ( $df = 225$ ) among individuals within populations. The variance among the established populations in the immediate vicinity of Volcán Lonquimay ( $N = 3$ ; pops. 3–5) is 8.6% ( $df = 2$ ; 95% CI = 6.4–10.7%); among the colonizing populations ( $N = 6$ ; pops. 8–13), it is 7.9% ( $df = 5$ ; 95% CI = 6.2–9.6%).

In a Bayesian analysis of the genetic variance among populations, the best approximation yielding the lowest DIC value was with the full model. For the 15 populations and using the full model (DIC value = 8936.3), the value of  $\theta$ -II (corresponding to  $\theta$ -B in previous versions of Hickory) is 0.125 (95% credible interval = 0.115–0.136). Among the established populations in the immediate vicinity of Volcán Lonquimay ( $N = 3$ ; pops. 3–5) and using the full model (DIC = 2038.5), the value of  $\theta$ -II is 0.073 (95% credible interval = 0.053–0.096). Among the colonizing populations ( $N = 6$ ; pops. 8–13) and using the full model (DIC = 3262.6), the value of  $\theta$ -II is 0.061 (95% credible interval = 0.040–0.076). DIC values obtained with the  $f$ -free model, which estimates  $\theta$  without estimating  $f$ , are not much higher than those obtained with the full model and values for  $\theta$ -II estimated by the  $f$ -free model are also very similar to those estimated by the full model (data not shown). The values for genetic differentiation among established populations and among colonizing populations obtained by AMOVA and Hickory analyses are very similar. Thus, established and colonizing populations in the immediate vicinity of Volcán Lonquimay have very similar levels of population differentiation.

A neighbor-joining clustering based on pairwise  $F_{ST}$  values among populations (Fig. 3) reveals the strongest separation between the two northern populations (pops. 1 and 2) and the other populations (all populations in the Lonquimay and surrounding area as well as the southern population; pops. 3–15). These results are consistent with those obtained by Bayesian clustering (Fig. 3), which assigns the individuals of the two northern populations (pops. 1 and 2) to one group (blue). Individuals of the populations in the Lonquimay and surrounding area (pops. 3–14) are intermixed in two groups (green and red). The southern population (pop. 15) is in a separate group (yellow), but some individuals in the Lonquimay and surrounding area are also assigned to this yellow group.

**Population characteristics**—When comparing population characteristics of the six established and seven colonizing populations in the Lonquimay and surrounding area, colonizing populations (pops. 8–13A) are smaller in size and occupy a smaller area than the established populations (pops. 3–7), though these differences are not statistically significant (Table 3). Population 14 on ash from an older eruption occupies a large area, similar to established populations. The two northern populations (pops. 1 and 2) are comparatively small in size and area, whereas the southern population (pop. 15) is very large.

In the vegetative growth category, the colonizing populations (pops. 8–13A) are clearly more vigorous than established

TABLE 2. Estimates of divergence of populations and within-population genetic diversity based on AFLP analysis from 16 individuals in each of 15 populations of *Nassauvia lagascae* var. *lanata*. The Mann–Whitney *U* test was used to assess the significance of differences between established and colonizing populations in the Lonquimay and surrounding area. Significant differences are seen in number of private bands and rarity index.

Region	Population	Estimates of divergence		Estimates of variation		
		Number of private bands	Rarity index	Total number of bands	Percentage of polymorphic bands	Shannon diversity index
North						
	1: Chillán	13	2.0	114	32.9	26.3
	2: Copahue	5	1.1	96	26.1	20.5
	Mean (±SD)	9.0 (±5.7)	1.6 (±0.6)	105.0 (±12.7)	29.5 (±4.8)	23.4 (±4.1)
Volcán Lonquimay and surrounding area						
Established populations						
	3: Cerros de Lanco	2	1.1	123	35.5	27.9
	4: Tolhuaca	8	1.9	163	49.8	38.1
	5: Colorado	9	2.0	168	53.4	41.9
	6: Sierra Nevada	2	1.1	127	39.4	32.6
	7: Pino Hachado	4	1.3	129	40.1	29.1
	Mean (±SD)	5.0 (±3.3)	1.5 (±0.4)	142.0 (±21.6)	43.6 (±7.6)	33.9 (±6.0)
Colonizing populations (since eruption of cone Navidad, December 1988)						
	8: Lonquimay	0	0.8	111	31.6	22.4
	9: Lonquimay	1	0.7	112	32.9	23.6
	10: Lonquimay	2	1.1	138	40.7	30.3
	11: Lonquimay	2	1.2	135	40.4	32.9
	12: Lonquimay	0	0.8	118	36.2	26.7
	13: Lonquimay	1	1.0	135	42.0	31.9
	Mean (±SD)	1.0 (±0.9)	0.9 (±0.2)	124.8 (±12.5)	37.3 (±4.4)	28.0 (±4.4)
Population growing on ash from an older eruption of Volcán Lonquimay						
	14: Lonquimay	2	0.7	106	30.9	24.2
South						
	15: Villarrica	9	1.9	128	38.1	30.5
Mann–Whitney <i>U</i> test						
	<i>Z</i>	–2.441	–2.373	–1.098	–0.913	–1.461
	(2-tailed significance)	(0.015)	(0.018)	(0.272)	(0.361)	(0.144)

populations (pops. 3–7) as shown by the parameters diameter of plants, height of plants, and number of shoots per individual (Table 3; differences are statistically significant at the 0.05 level). Population 14 on ash from an older eruption is in the range of established populations in terms of diameter. The northern populations (pops. 1 and 2) and the southern population (pop. 15) are similar to established populations of the Lonquimay and surrounding area with respect to vegetative vigor.

In the reproduction category, the proportion of reproductive individuals in the populations and the number of flowering shoots per reproductive individuals are not significantly different among established (pops. 3–7) and colonizing populations (pops. 8–13A; Table 3). Population 14, the northern populations (pops. 1 and 2) and the southern population (pop. 15) also have similar values.

Regarding the occurrence of seedlings in the populations, in five of the eight colonizing populations on Volcán Lonquimay (pops. 8–12), some seedlings were observed close to their presumed mother plants; no seedlings were observed for the other two colonizing populations (pops. 13 and 13A) and for population 14, but populations 13 and 13A had some 1-yr-old plants. Similar observations were made for five of the six established populations sampled here (observations were not made for pop. 3). This feature varied from many seedlings (pop. 4) to no seedlings (pop. 7) with populations 5, 6, and 6A having some seedlings close to their presumed mother plants. In the northern populations (pops. 1 and 2) and the southern population (pop. 15), no seedlings were observed.

In the general vegetation category, the coverage of the herb layer is significantly less in the colonizing populations (pops.

8–13A) than in the established populations (pops. 3–7; Table 3). Population 14 has a low coverage like colonizing populations. In the northern populations (pops. 1 and 2) and the southern population (pop. 15), the coverage is similar to that of established populations. To the contrary, the height of the herb layer is similar in established and colonizing populations of the Lonquimay and surrounding area as well as in all other populations.

## DISCUSSION

**Effect of colonization on genetic diversity in *Nassauvia lagascae* var. *lanata***—We consider two aspects of the effect of colonization on genetic diversity, first the within-population component and second the among-population component ( $F_{ST}$ ). A significant reduction of the number of private bands and rarity index in colonizing populations in comparison to established populations suggests that there indeed was a founder effect because rare alleles have not been transmitted by founding propagules (see Nei et al., 1975; we have to keep in mind, however, that only 16 individuals have been sampled per population and that inability to detect private and rare bands could also result from sampling error). Colonizing populations also have reduced levels of within-population variation (as measured by the total number of bands, percentage of polymorphic bands, and Shannon diversity index) in comparison to established populations, although this reduction is not statistically significant.

Genetic differentiation ( $F_{ST}$ ) among colonizing populations, however, is not higher than among established populations in the immediate vicinity of Volcán Lonquimay (pops. 3–5), as

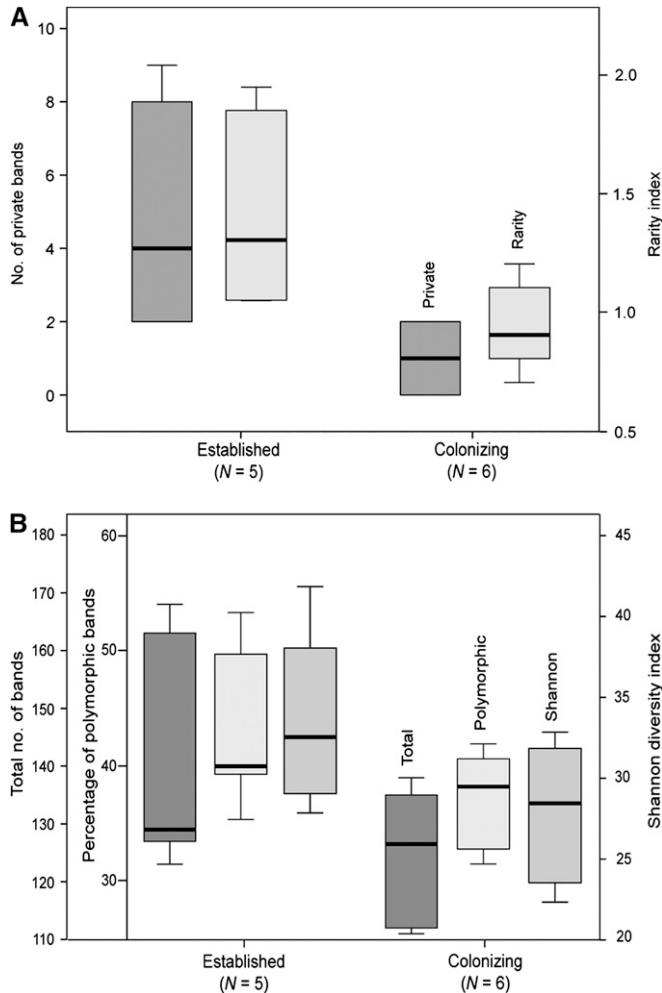


Fig. 2. Box plots showing the median, 25% and 75% quartiles (box), and non-outlier range (whiskers) of (A) number of private bands and rarity index and (B) estimates of genetic variation based on AFLP data for the five established and six colonizing populations of *Nassauvia lagascae* var. *lanata* of the Lonquimay and surrounding area.

would be expected with a founder effect. However, the number of generations needed to counterbalance a founder effect is unknown (see Pannell and Dorken, 2006), so that, alternatively, an eventual reduction of  $F_{ST}$  brought about by an initial founder effect could have been already abolished through population growth and immigration in subsequent years. If the newly exposed areas were colonized repeatedly from the surrounding populations, this would reduce  $F_{ST}$ . Thus, our results indicate that 14 yr after creation of the vacant site a founder effect is still recognizable within populations as a significant reduction of rare alleles and a moderate reduction of within-population variation, but no longer at the among-population level.

**Genetic diversity in relation to biological characteristics of the populations**—Our estimates of the total number of individuals in the populations indicate that colonizing populations are still rather small in comparison to established populations. Similarly, the area occupied by colonizing populations is still smaller than that of established populations.

Plants of *N. lagascae* var. *lanata* in the colonizing populations are significantly larger in their overall size than in the

established populations (Table 3) indicating that the species is well adapted to the volcanic environment. We were not able to detect any significant differences, however, in reproductive features of plants in colonizing vs. established populations. The time from seedling establishment to first flowering and the periodicity of flowering in *N. lagascae* var. *lanata* are unknown. The plants are not expected to flower in their first year, as we have observed juvenile, probably 1-yr-old plants, which were not yet in their reproductive stage. However, the majority of plants in the colonizing populations seem to have reached their reproductive stage.

*Nassauvia lagascae* var. *lanata* also seems to be poorly adapted to dispersal over long distances. The great majority of fruits and seedlings remain in the immediate vicinity of the presumed mother plants (K. Tremetsberger, personal observation). The presence in this species of a pappus with deciduous bristles, plus a fruit with a waxy covering and lack of structures that facilitate dispersal, suggest that wind is not a relevant factor in dispersion. Diaspores appear to be dispersed via hydrochory involving water from rain or melting snow. Thus, secondary dispersal would have a greater importance, as also found in *Azorella madrepora*, *Madia sativa*, *Nassauvia pinnigera*, and *Pozoa coriacea*, all of which are species of the altoandine habitat (Castor, 2002). Considering the secondary dispersal of *N. lagascae* var. *lanata* via running water, immigration into newly available areas should come from survivor populations growing near the edges of the affected areas. Dispersal over longer distances (i.e., from different volcanoes and mountains), possibly through zoochory, is expected to be very rare. In coincidence with this expectation, the neighbor-joining tree reveals genetic affinities of the colonizing populations with population 14 on ash from an older eruption and established populations in the immediate vicinity of Volcán Lonquimay (pops. 3–5) rather than with populations further away (pops. 6 and 7).

Our results show that the time elapsed from the creation of the new vacant habitat by the volcanic eruption to the date of sampling was not sufficient to restore genetic diversity within colonizing populations. Population 14 growing on ash from an older eruption has also not recovered within-population genetic diversity yet. Reasons for this might include slow population growth, kin-structure within populations, and infrequent long-distance dispersal events.

**Broader geographical patterns in the southern Andes**—To interpret groupings of populations of the Bayesian clustering and neighbor-joining tree on a broader geographical scale, it is necessary to consider the Pleistocene history of the region. During the last glacial maximum (c. 18–20 kyr BP), the southern Andes were covered by a very large, continuous ice sheet, which reached the Araucanía region at its northern end (e.g., Singer et al., 2004; Rabassa 2008). North of the continuous ice sheet, local glaciers of decreasing size were developed. Groupings of populations of *N. lagascae* var. *lanata* revealed by Bayesian clustering and the neighbor-joining tree reflect this situation and parallel results obtained for other herbaceous species growing in the same area. For example, in *Hypochaeris tenuifolia*, the northern populations (including Chillán and Volcán Antuco) are distinct from a large group including all populations from the Lonquimay and surrounding area as well as populations further south (Volcán Villarrica and Volcán Lanín; Tremetsberger et al., 2003). This suggests that Pleistocene refugia of the altoandine vegetation isolated by glacial tongues were located north of the Araucanía region in the southern Andes, resulting in genetic distinctness of

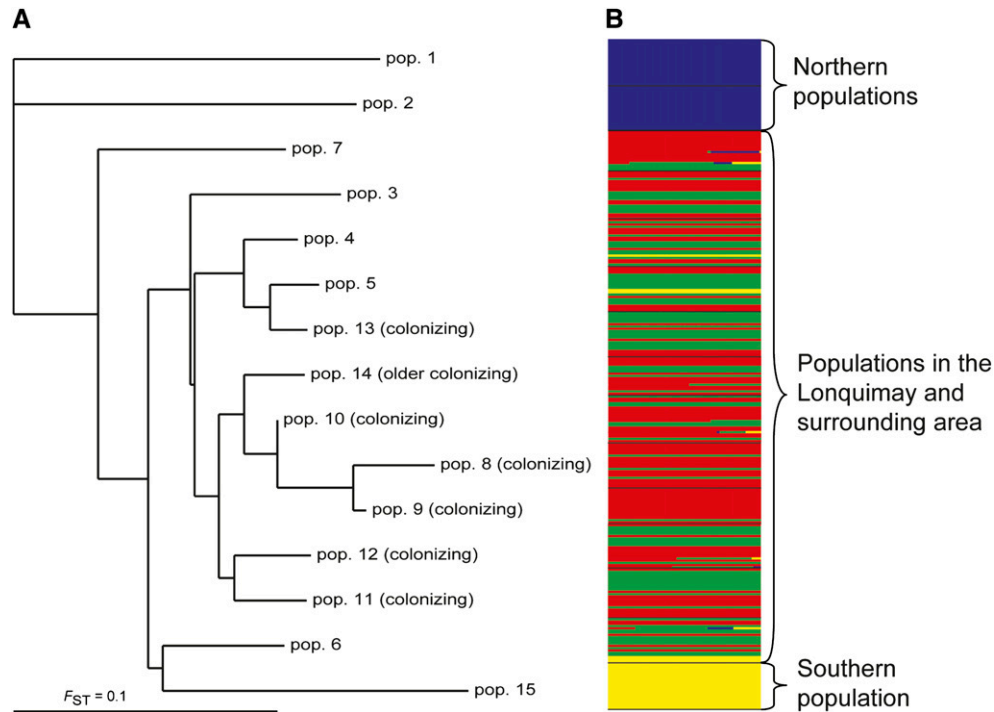


Fig. 3. Geographical structure among populations of *Nassauvia lagascae* var. *lanata*. (A) Neighbor-joining clustering based on pairwise  $F_{ST}$  values among populations; bootstrap support is <50% for all bifurcations (obtained by subjecting the genetic [Nei and Li, 1979] distance matrix among individual AFLP phenotypes to a neighbor-joining analysis and running 500 replicates). (B) Population structure inferred by Bayesian clustering ( $K = 4$ ).

the refugial populations (e.g., in Chillán, Copahue, and Antuco). From the Araucanía region southward, the Andes were recolonized in the Holocene from adjacent refugia, resulting in a rather homogeneous genetic composition of populations.

**Differences in patterns of colonization in plant species**— Two early colonizers of volcanic ash fields in the southern Andes, *Nassauvia lagascae* var. *lanata* and *Hypochaeris tenuifolia*, both belonging to Asteraceae, have been studied. In *H. tenuifolia*, genetic diversity within and among established and colonizing populations has also been investigated by means of AFLP on the volcanic ash fields resulting from the December 1988 eruption of the Navidad cone (Tremetsberger et al., 2003). The results indicated that population differentiation was lower among colonizing populations (collected 10 years after the eruption) than among other populations immediately and distantly outside the zone of disturbance. The genetic diversity within the colonizing populations did not differ significantly from that of the established populations. The closest genetic similarity occurred between colonizing and nearby established populations as well as populations in adjacent southern regions (Araucanía Region).

Thus, the effect of colonization on the distribution of genetic diversity within and among populations in the two perennial and probably outcrossing plants *N. lagascae* var. *lanata* and *H. tenuifolia* is different, and these differences should relate to biological characteristics of the species. One major difference refers to dispersal capabilities, especially the ability to disperse over long distances. In *N. lagascae* var. *lanata*, whose offspring frequently remain in the close vicinity of the presumed mother plant and with long-distance dispersals being probably rare events, immigrants to the newly available areas are probably few and recruited from the immediately surrounding areas.

Population growth is also slow, enabling only a gradual development of within-population diversity. In contrast, *H. tenuifolia* possesses a well-developed and permanent pappus, which allows the primary dispersal of the fruit by wind far away from the parental plant (Andersen, 1993), probably including occasional among-site dispersal in the island-like altoandine habitats of the southern Andes. Immigrants of *H. tenuifolia* to the newly available areas are probably many and recruited from different source populations (Tremetsberger et al., 2003). Dispersal within populations should also be much more pronounced in *H. tenuifolia*, counteracting pronounced kin-structuring in populations. In addition, *H. tenuifolia* has a strong capacity to propagate vegetatively via underground stolons. Population growth in *H. tenuifolia* is fast, with the colonizing populations having already exceeded the established populations in number of individuals only 10 years after the eruption. In summary, therefore, there is evidence for an additional component to gene flow brought about through colonization in *H. tenuifolia*, but not in *N. lagascae* var. *lanata*, where we find evidence for a founder effect. In other words, the time scale for recovery of population sizes and within-population genetic diversity is fast in *H. tenuifolia* and slow in *N. lagascae* var. *lanata*.

Evidence for a founder effect such as inferred for *N. lagascae* var. *lanata* has also been found in other species, such as in *Lupinus lepidus* (Fabaceae; J. G. Bishop, Washington State University, and R. J. Dyer, Virginia Commonwealth University, unpublished data) and in the two dioecious plants *Silene alba* (Caryophyllaceae; McCauley et al., 1995) and *S. dioica* (Giles and Goudet, 1997). Bishop and Dyer (unpublished data) examined the population genetic consequences of colonization in *Lupinus lepidus* var. *lobbii* on areas newly covered with ash of Mount St. Helens (Washington, USA) after its eruption on

TABLE 3. Population characteristics of *Nassauvia lagascae* var. *lanata*. The Mann-Whitney *U* test was used to assess the significance of differences between established and colonizing populations in the Lonquimay and surrounding area. Significant differences at the 0.05 level are seen in the three categories of vegetative growth (diameter, height, and number of shoots of plants) and coverage of herb layer; nd = no data.

Region	Size of population		Vegetative growth			Reproduction		General vegetation		
	Population	Total no. individuals	Area (m <sup>2</sup> )	Mean diameter of plants (cm)	Mean height of plants (cm)	No. shoots per individual	Proportion of reproductive individuals	No. flowering shoots per reproductive individual	Coverage of herb layer (%)	Height of herb layer (cm)
North										
	1	40	1500	6.0	2.5	8.5	0.7	3.0	10.0	6.8
	2	300	2500	7.0	2.0	9.0	0.9	5.0	5.0	2.6
	Mean (±SD)	170 (±184)	2000 (±707)	6.5 (±0.7)	2.3 (±0.4)	8.8 (±0.4)	0.8 (±0.2)	4.0 (±1.4)	7.5 (±3.5)	4.7 (±3.0)
Volcán Lonquimay and surrounding area										
Established populations										
	3	50	1000	nd	nd	10.0	0.9	3.0	4.0	3.0
	4	3000	6000	3.0	1.0	8.0	0.3	3.0	2.0	4.4
	5	100	5000	7.0	1.8	14.5	0.8	1.0	25.0	1.9
	6	500	10000	5.0	2.0	8.5	0.5	3.0	1.0	6.5
	6A	1000	3000	9.0	2.5	23.0	0.8	2.0	3.0	10.5
	7	1000	30000	6.0	3.0	10.0	0.7	3.0	10.0	4.0
	Mean (±SD)	942 (±1090)	9167 (±10647)	6.0 (±2.2)	2.1 (±0.8)	12.3 (±5.7)	0.7 (±0.2)	2.5 (±0.8)	7.5 (±9.1)	5.1 (±3.1)
Colonizing populations (since eruption of cone Navidad, December 1988)										
	8	50	1000	11.0	5.0	21.5	0.9	5.5	0.5	4.0
	9	250	5000	10.0	4.0	24.5	0.9	8.0	0.2	3.5
	10	150	1500	13.0	3.0	48.0	0.5	6.5	0.8	4.0
	11	50	1250	6.0	2.0	9.0	0.5	2.0	0.5	4.3
	12	100	3000	12.0	4.5	35.0	0.8	2.5	0.5	7.8
	13	540	1800	10.0	4.0	31.0	0.8	3.0	1.5	5.5
	13A	300	1500	10.0	4.0	43.0	0.8	3.5	1.5	5.5
	Mean (±SD)	206 (±176)	2150 (±1411)	10.3 (±2.2)	3.8 (±1.0)	30.3 (±13.3)	0.7 (±0.2)	4.4 (±2.3)	0.8 (±0.5)	4.9 (±1.5)
Population growing on ash from an older eruption of Volcán Lonquimay										
	14	300	7500	6.0	3.0	17.0	0.5	3.0	0.5	5.1
South										
	15	100000	100000	5.0	2.0	12.0	0.5	1.0	10.0	7.9
Mann-Whitney <i>U</i> test										
	Z	-1.368	-1.796	-2.458	-2.380	-2.289	-0.436	-1.545	-2.733	-0.288
	(2-tailed significance)	(0.171)	(0.073)	(0.014)	(0.017)	(0.022)	(0.663)	(0.122)	(0.006)	(0.774)

18 May 1980 based on size polymorphism of PCR products in two loci. They found a very strong founder effect with an increase of  $F_{ST}$  from 0.02 in surviving populations to 0.26 in newly founded populations (maximum 4 years old). Newly founded populations of *S. alba* (maximum 4 years old) showed only a small increase in  $F_{ST}$  (0.20) in comparison to older populations (0.13; McCauley et al., 1995). Similarly, *S. dioica* also showed a considerable increase of  $F_{ST}$  in younger populations (age < 30 yr, size < 4000 individuals,  $F_{ST}$  = 0.06) in comparison to intermediate populations, which had the highest genetic and demographic equilibrium in this system (30 yr < age < 280 yr, size > 4000 individuals,  $F_{ST}$  = 0.03; Giles and Goudet, 1997). All of these species have low dispersal abilities similar to *N. lagascae* var. *lanata* (e.g., see Ingvarsson and Giles, 1999, for *S. dioica*). For a single island population of *S. dioica* less than 10 yr old in the Skeppsvik Archipelago, Umeå, Sweden, Ingvarsson and Giles (1999) presented evidence for kin-structured colonization resulting in as high or higher levels of genetic differentiation in the colonizing population in comparison to what is observed over larger scales in the archipelago. Similarly, Jacquemyn et al. (2009) investigated the genetic diversity in subsequent generations of a founding population of *Primula elatior* (Primulaceae) to understand the processes that affect them after the settlement. They found that genetic diversity increased sub-

stantially from the first generation to the second and third generations. *Primula elatior* is a species with limited seed dispersal similar to *N. lagascae* var. *lanata*. The authors found that seedling survival often occurs at particular microsites that are not heavily affected by competition leading to a highly clustered distribution pattern within the population. We hypothesize that kin structure is also an important factor for slow increase of genetic diversity after colonization in *N. lagascae* var. *lanata*.

Contrasting results (i.e., an additional component to gene flow resulting from the colonization process) have been found in other colonizing species in addition to *Hypochaeris tenuifolia*, such as *Spartina alterniflora* (Poaceae; Travis et al., 2002) and *Vaccinium membranaceum* (Ericaceae; Yang et al., 2008). In *S. alterniflora* growing in restored vs. natural wetlands, the genetic diversity in the restored population was as high as in the natural marsh populations (Travis et al., 2002). In the animal-dispersed *V. membranaceum* growing on volcanic deposits of Mount St. Helens (Washington, USA), genetic diversity in the newly founded population 24 yr after the eruption was higher than in most of the source regions, suggesting a lack of a strong founder effect (Yang et al., 2008). Similar to *H. tenuifolia*, high gene flow among sources and long-distance dispersal were inferred to be important processes shaping the genetic diversity in the young *V. membranaceum* population.



In conclusion, this study demonstrates the importance of combining genetic, ecological, and demographic investigations in populations to understand better how colonization shapes the genetic structure of populations. It is clear that the effect of colonization can be in opposite directions (i.e., additional gene flow vs. founder effect) in different species depending on the species' biological characteristics. Most importantly, the time frame needed for populations to reach equilibrium after colonization can be very different and is poorly understood. It would be advisable for future studies to include population surveys in several subsequent time intervals on the same populations after colonization to trace the development of genetic diversity within and among populations in relation to population characteristics over time.

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