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ORIGINAL ARTICLE

Genetic diversity of pioneer populations: the case of *Nassauvia argentea* (Asteraceae: Mutisieae) on Volcán Lonquimay, Chile

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Abstract Colonising populations do not always exhibit founder effects. Common explanations are high levels of immigration and/or reproduction, but few empirical tests have been done. We measured genetic diversity of *Nassauvia argentea* in terms of variation and divergence of plant populations that have colonised Volcán Lonquimay, Chile, following its latest eruption in 1988. Fifteen individuals from each of ten populations were analysed using amplified fragment length polymorphism (AFLP) markers. Genetic variation and divergence were lower in colonising populations than established ones, but not significantly so (ANOVA and Kruskal-Wallis tests, $p < 0.05$). No consistent or significant trends were obtained from regressions with demographic variables. Bayesian analysis of population structure reveals close relatedness among populations of all ages on the volcano. We concluded that no conspicuous founder effect has occurred in the genetic diversity of populations colonising a newly derived volcanic environment. An important implication of this is the role of proximity to source regions and species vigour in moulding genetic diversity of colonisers from different species.

Keywords AFLPs · Colonisation · Compositae · Founder effect · Volcanic disturbance

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Introduction

Plant populations colonising fresh landscapes can provide insights into patterns of dispersal and genetic diversity. In this time of extensive habitat change, it is especially appropriate to examine processes that structure populational genetic diversity in early succession after catastrophic disturbances. One key concept is the founder effect, which has a long history (Provine 1989; Mayr 1942; Carson 1968; Slatkin 1977; Templeton 1980; Wade and McCauley 1988). The broad reasoning is that few individuals successfully colonise, leading to genetically depauperate colonising populations and potential for evolution of novel forms out of this bottleneck. The concept is often invoked as a valid model for inference of past processes from modern genetic data in phylogeographic studies (Deshpande et al. 2009). Many have queried the founder effect concept in terms of speciation (Barton and Mallet 1996; Charlesworth 2003; Coyne and Orr 2004; Comes et al. 2008), including observations that cast doubt on declines in genetic diversity (Whittaker and Fernández-Palacios 2008). In this article we focus on the genetic diversity of colonising populations.

Significant losses of genetic diversity have been observed in colonising populations (Polans and Allard 1989; McCauley et al. 1995; Merilä et al. 1996; Robichaux et al. 1997; Després et al. 2002; Jacquemyn et al. 2008), and population genetic modelling substantiates these findings (Nei et al. 1975; Chakraborty and Nei 1977; Maruyama and Fuerst 1985). Yet contrary to founder effect theory, there are studies that highlight a swift recovery of genetic diversity after a population bottleneck (Merilä et al. 1996; Keller et al. 2001; Travis et al. 2002; Honnay et al. 2009), some work even suggesting that molecular variation in founder populations can even exceed that of source populations (Carson and Wisotzkey 1989).

Questions regarding the legacy of colonisation need further investigation to establish the basis on which much modelling and theoretical reasoning is based. The variety of findings has led some to question the validity of the founder effect concept (Barton and Charlesworth 1984; Leberg 1992; Barton and Mallet 1996; Hamrick and Nason 1996). The occurrence of a founder effect, however, relates to the size of the founding population, subsequent gene flow, and the rate of population growth (Slatkin 1977; Merilä et al. 1996; Sun 1996). Founder effects are valid expectations only where colonisation is minimal and population growth is slow. Counterexamples in the literature, therefore, may simply result from these ecological conditions not being satisfied, rather than any flaw in founder effect theory per se. Demographic variables can add analytic power when studying founder effects (López et al. 2010), but studies of neutral genetic diversity following a bottleneck rarely include population characteristics in their analysis.

Volcanic eruptions create an ideal opportunity to study genetic diversity in colonising populations. Colonisations of species and their extinctions during the course of volcanic successions have been well studied, and they exemplify slow colonisation (e.g. Fridriksson and Magnusson 1992; Thornton 1996, 2001; del Moral and Lacher 2005), consistent with founder effect expectations. However, only a handful of post-eruption studies of genetic diversity have been conducted. Parrish (2002) found no reduction in genetic diversity in five tree species on Krakatau, following the last major eruption in 1883. Founder effects are likely to be most profound in the early years, when little immigration, recombination or mutation has occurred. Genetic diversity of *Vaccinium membranaceum* has been studied 24 years after the 1980 eruption of Mount St. Helens, USA, by Yang et al. (2008), who found high levels of gene flow from many sources and little support for any founder effect. On a shorter timescale, sampling of two Asteraceae species following 25 December 1988 eruption on Volcán Lonquimay, Chile, revealed no founder effect in one case (Tremetsberger et al. 2003) and ongoing genetic rescue in the other (López et al. 2010).

Here we study one plant species on and around Volcán Lonquimay, southern Chile, which last erupted in 1988–1989. We examine how well the population genetics of *Nassauvia argentea* (Asteraceae: Philippi 1894) relates to founder effect theory in three ways: (1) contrast the genetic variation and divergence of recent colonisers of *N. argentea* with established populations, (2) explore possible relationships with demographic characteristics and (3) examine the genetic affinities of populations on the volcano. If a founder effect occurred during colonisation, we expect clear reductions in within-population genetic variation in the colonising populations and alterations of the frequency distribution of alleles in favour of more common variants (Pannell and Dorken 2006). Moreover, if populations were founded by individuals from a single source population or if few founding individuals were drawn from different source populations, we expect an increase in genetic differentiation among populations. Only if a high number of colonists from different source populations exists do we expect a decrease in genetic differentiation (see Pannell and Dorken 2006 for a review).

Materials and methods

Species

Nassauvia argentea is a perennial, rhizomatous herb with stems 10–25 cm long (sometimes shorter than 10 cm; KT, personal observation) and covered with imbricated, curved leaves (Cabrera 1982). The plants usually form dense or seldom loose cushions, and do not propagate vegetatively.

They have long, lateral roots for anchorage in the volcanic ash. Flowering heads are condensed in secondary heads, 12–20 mm in diameter. Individual flowers are white, with petals 6.5 mm long. Achenes are glabrous; the pappus consists of a few deciduous linear, white bristles. The mode of dispersal of *Nassauvia* species is not obvious, but it might be via small lizards or birds (Castor 2002). Frequently, fruits also remain in the heads; these bend down towards the soil and release the fruits in the immediate vicinity of the mother plant (KT, personal observation). A self-incompatible breeding system has been reported for several *Nassauvia* species (Arroyo and Squeo 1990), but no experiments have yet been carried out to determine the breeding system of *N. argentea*. Diptera have been reported as the most frequent flower visitors in *Nassauvia* species, followed by Hymenoptera and Lepidoptera (Arroyo et al. 1982). The habitat is stony soil or volcanic ash 1,500–1,960 m above sea level in the Andes of Chile and Argentina (Cabrera 1982; KT, personal observation).

Sampling

A test of founder effects can be undertaken by comparing genetic diversity (in terms of genetic variation and genetic divergence) of colonising populations with that of older populations surrounding the volcano. During the last major eruption, lava flowed northeast from the Navidad cone of Volcán Lonquimay from 25 December 1988 until 25 March 1989, and 625 million m^3 ash was deposited south and westwards (Fig. 1; González-Ferrán 1995). Prior to this, the last recorded eruption of Lonquimay was in 1940. A handful of populations has colonised the Navidad ashfield since 1988.

During the austral summers 2002 and 2003, i.e., 13–14 years after the eruption, we sampled five populations colonising the ash (populations 1–5), one surviving population at the edge of the ashfield, which was affected by ash falls of the eruption, but not to an extent that it was completely eradicated (population 6), and four established populations (two around Lonquimay and two to the south and east, populations 7–10, Fig. 1; Table 1). Assuming colonisation immediately after the eruption in austral spring 1989 and first fruit production in austral summer 1991 (when first colonisers were 2 years old), the maximum possible number of generations until sampling would be 12 or 13. Volcán Lonquimay is in the Araucanía region of Chile, but sampling extended east across the border into the Neuquén region of Argentina (Paso Pino Hachado). Individuals were selected randomly from each population and placed in silica gel. Vouchers of each sampled population are deposited in the University of Vienna (WU) herbarium.

The following variables were recorded to investigate possible relations between demography and genetic diversity: total number of individuals, area covered, mean plant diameter, mean plant height, number of shoots per individual, proportion of fruiting or flowering (i.e., reproductive) individuals, number of flowering shoots per reproductive individual, percentage of herb cover and herb layer height (Online Resource 1). Population density was calculated by dividing the number of individuals by the area covered by the population. Missing data occurred as follows: population 2, mean plant diameter; populations 5, 9 and 10 area, size, density; population 8 mean height of vegetative shoots.

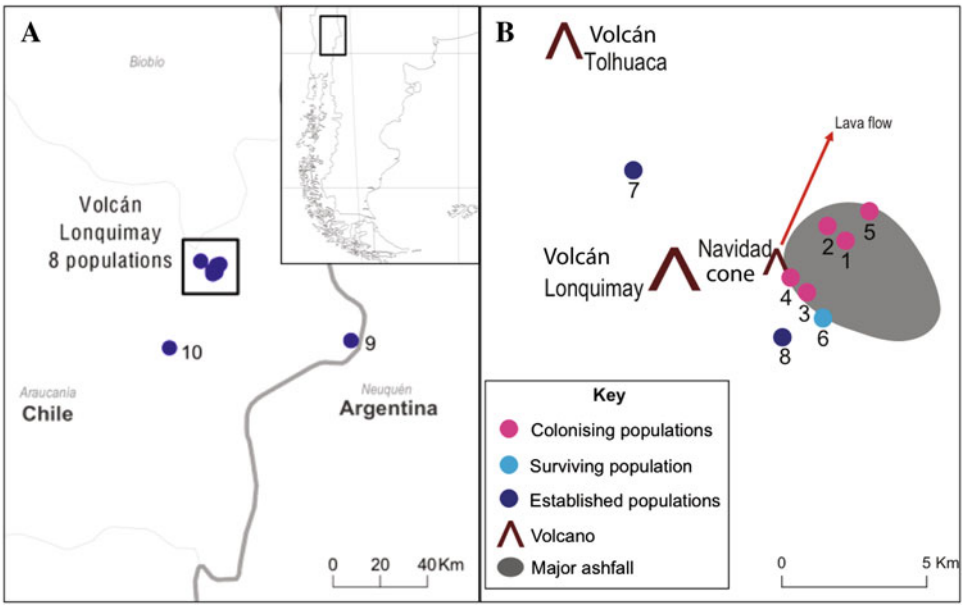

Fig. 1 Maps indicating populations of *Nassauvia argentea* sampled in Chile for AFLP analysis. **a** Area of study. **b** Detail of Lonquimay area and major ashfall of Navidad cone

Table 1 Populations of *Nassauvia argentea* sampled for AFLP analysis

Population number	Location	Collection number	Latitude	Longitude	Elevation (m)
Colonising populations					
1	Navidad ash	24	38°21′50″ S	71°31′37″ W	1,905
2	Navidad ash	92	38°21′32″ S	71°32′02″ W	1,785
3	Navidad ash	1,043	38°22′59″ S	71°32′36″ W	1,930
4	Navidad ash	96	38°22′49″ S	71°33′13″ W	1,930
5	Navidad ash	82/1,068	38°21′15″ S	71°30′59″ W	1,840
Surviving population					
6	Lonquimay old ash	1,046	38°23′12″ S	71°32′17″ W	1,960
Established populations					
Near Lonquimay					
7	Path to Tolhuaca	1,086	38°20′30″ S	71°36′31″ W	1,830
8	Lonquimay Skilift	1,054	38°23′39″ S	71°33′13″ W	1,640
South					
9	Paso Pino Hachado	1,042	38°39′30″ S	70°53′50″ W	1,960
10	Volcán Llaima	108	38°41′19″ S	71°46′48″ W	1,910

Vouchers are deposited at WU. Collected by KT, AJ and SG

Amplified fragment length polymorphism

One hundred fifty samples of *N. argentea* from ten populations were analysed for AFLP data, with ten replicates. This method provides presence/absence data for many dominant neutral bands sampled randomly from across the genome (Mueller and Wolfenbarger 1999). Genomic DNA was extracted from silica-gel dried leaf material via the CTAB method (Doyle and Doyle 1987) with minor modifications (Tremetsberger et al. 2003). The AFLP procedure developed by Vos et al. (1995) was followed, with modifications detailed in Tremetsberger et al. (2003). A primer trial with ten individuals led to three selective combinations being used: *EcoRI*-ACA/*MseI*-CTAG, *EcoRI*-AAG/*MseI*-CTTC and *EcoRI*-ACC/*MseI*-CATA. AFLP analysis was replicated for ten samples. Quality control of electropherograms after size-calling with GeneScan (Applied Biosystems Inc., 850 Lincoln Center Driver, Foster City, CA, v3.7.1) was undertaken in Genographer (Benham 2001, v1.6.0). Low intensity and noisy electropherograms were omitted until an equal number of good quality lanes remained in each population.

Manual scoring can lead to operator bias (Bonin et al. 2007), but fully automated scoring eliminates this. AFLP scoring used GeneMarker (SoftGenetics, LLC, State College, PA, v1.85) for both fragment length estimation and conversion to binary data (Miller et al. 2007). As use of automated scoring in population level analysis of AFLPs is very new, the options used in automatic scoring are detailed as follows. An automatic panel of all scorable bins was generated (Curtin et al. 2007). Our procedure for optimising scoring parameters is an expanded version of that of Holland et al. (2008). Minimum fragment length and peak intensity were optimised on a representative sample of 30 individuals across all populations from 50 to 500 bp. The range of peak height intensity is extended from Holland et al. (2008), in intervals of 10 from 50 to 200 rfu. In a finding similar to Holland et al. (2008), altering the Stutter Peak Filter and Local and Global Detection led to little difference in scoring during optimisation and was left off for all further analysis. Review criteria were set to "fail $<$ 1 check $<$ 1 $<$ pass". All other parameters were kept at default.

The information content per bin metric (I_{bin}, Arrigo et al. 2009) was calculated to produce a shortlist of optimal combinations of minimum fragment length and peak height parameters. I_{bin} measures pair-wise differences between individual scoring outputs relative to the total number of bands scored. The higher the I_{bin} is, the greater the contribution of each scored band to genetic diversity measures. Parameter settings with the top five I_{bin} scores were used in automatic scoring of the entire final data set. To verify consistency in results, five neighbour-joining trees were constructed in PAUP* (Swofford 2002, v4.0) using Nei-Li distance (following Wooten and Tolley-Jordan 2009): similar structures were revealed, except for the run with higher intensity (150 rfu). From the remaining four data sets with optimal I_{bin}, the parameter combination yielding a relatively low mismatch error rate between replicates (Sokal and Michener 1958, cited in Bonin et al. 2007), and a high number of bands was used for all subsequent analysis. Thus, peak height threshold used in final scoring was 60 rfu and minimum fragment length 50 bp. High quality size calibrations (>80% accuracy, linear trend) were obtained for all samples.

Data analysis

Genetic diversity within populations

The aspects of genetic diversity measured here reflect variation of alleles within populations as well as divergence of populations in terms of rare alleles they may contain. Genetic variation per population was measured by the total number of bands, the proportion of polymorphic bands and the Shannon index of diversity. Genetic divergence was measured by the number of private bands and the rarity index. The number of phenotypes, percentage of polymorphic loci and rarity index for each population was calculated in R (R Development Core Team 2008, v2.1.8) using the AFLPdat script (Ehrich 2006, July 2008 release). The Shannon diversity index [$H_{Sh} = -\sum p_i * \ln(p_i)$] was calculated from AFLPdat output, with p_i being the proportion of occurrences for *i*th band in each population relative to the total number of bands. The number of Private Bands was calculated in FAMD (Schlüter and Harris 2006, v1.21).

Correlation between all variables was tested using the Pearson method as default, reverting to the Spearman method when either variable was not normally distributed [$p < 0.05$, Anderson–Darling test, R nortest package (Gross 2006, v1.0)]. Population type (colonising/surviving/established) was classed as a factor, all other variables as numeric or integer. ANOVAs were used to test whether genetic diversity metrics were significantly lower in colonising populations (Kruskal-Wallis test used when the genetic diversity metric was not normally distributed).

Demographic data were missing for some variables for some populations, so analysis was grouped by omitting any population with missing data for those affected variables. Additive and interactive linear regression models were constructed in R for all permutations of independent variables. While a few variables were not normally distributed, nonparametric regression requires large sample sizes to estimate parameters from the dataset itself. Given the general robustness and greater statistical power of parametric tests, this study proceeded with linear models. Checks were undertaken to ensure model residuals were normally distributed and that their variance was homogeneous.

Genetic structure among populations

Genetic differentiation within and between populations was assessed with a locus-by-locus Analysis of Molecular Variance (AMOVA) in Arlequin (Excoffier et al. 2006, v3.1), which assumes Hardy-Weinberg equilibrium. Since Hardy-Weinberg equilibrium is unlikely to be present in colonising populations, Bayesian modelling is necessary (Hutchison and Templeton 1999). To corroborate AMOVA results, F_{ST} was also estimated using Theta-II from Hickory (Holsinger et al. 2002, v1.1), this being the most reliable estimate of F_{ST} for describing genetic differentiation among small numbers of populations (Holsinger and Wallace 2004). The best fitting Bayesian model (from the full model, *f*-free model and $f = 0$ model) was assessed using the Deviance Information Criterion (Spiegelhalter et al. 2002). Bayesian clustering of individuals, where the number of populations is treated as unknown parameter, was conducted in BAPS (Corander et al. 2003, v5.2). The program chooses the number of populations and assigns individuals to these populations in a way that the joint posterior distribution of the population structure and the underlying allele or genotype frequencies of the respective populations are maximised. When using dominant markers, BAPS models the underlying population genotype frequencies instead of the allele frequencies considered for co-dominant markers (Corander et al. 2003). The maximum number of populations was ten for mixture analysis attempting to identify population structure by clustering individuals into genetically divergent groups. Subsequent admixture analysis aiming at separating the ancestral sources of the genome of different individuals (Corander and Marttinen 2006) was performed with default settings based on results of mixture clustering. A distance matrix was calculated from the AFLP matrix and used as input for the phylogenetic network with the NeighborNet algorithm (Bryant and Moulton 2004), as implemented in the software SplitsTree4 ver. 4.10 (Huson and Bryant 2006).

Results

Complete analysis of 15 individuals from each of 10 populations revealed 267 AFLP bands, of which 263 (98.5%) were polymorphic. All individuals had unique AFLP profiles. From ten (6.7%) replicated individuals, the mismatch error rate was 1.31%.

Genetic diversity within populations

There is a successive rise in the means of all genetic variation and divergence metrics from colonising to surviving to established populations (Table 2). However, values for each category do overlap (Fig. 2), making the difference not statistically significant. The lowest p value was 0.457 for the Shannon index (ANOVA $F_{2,7} = 0.879$). This indicates a very weak founder effect.

Because one common explanation for weak founder effects is fast population growth, we examined relationships between genetic diversity and demographic characteristics. From the linear models constructed for all diversity metrics with demographic characteristics (see

Table 2 Genetic variation and divergence metrics from AFLPs of *Nassauvia argentea* populations

Population number	Total number of fragments	Polymorphic bands (%)	Shannon index	Private bands	Rarity index
Colonising populations					
1	104	25.9	27.9	1	0.9
2	111	29.0	24.9	1	1.1
3	136	36.7	33.9	10	2.2
4	132	34.9	34.6	2	1.6
5	137	34.6	35.7	13	1.8
Mean	124.0	32.2	31.4	5.4	1.5
SD	15.4	4.6	4.7	5.7	0.5
Surviving population					
6	137	36.7	34.6	6	1.8
Established populations					
Near Lonquimay					
7	120	30.6	34.4	2	1.3
8	128	35.5	32.9	9	1.4
South					
9	161	45.7	44.9	37	3.9
10	119	29.9	32.1	9	1.6
Mean	132.0	35.4	36.1	14.3	2.1
SD	19.7	7.3	5.9	15.5	1.2

Online Resource 1), no consistent or strong trends were found. From 50 regressions undertaken (five diversity metrics, 10 demographic variables), only one model was statistically significant ($p < 0.05$): the percentage of herb cover was significantly positively related to the number of private bands ($t = 2.63$, $p = 0.029$, Adj $R^2 = 0.402$). In other models there was no consistent positive trend; indeed, all five models incorporating population size had negative coefficients, though these were not statistically significant.

Genetic structure among populations

AMOVA attributed 9.36% variance among all ten populations (95% CI 7.53–11.26%) and 90.64% among individuals within populations. Variance was highest among established populations (11.89%, $N = 4$, populations 7–10, 95% CI 8.86–14.99%). Variance among the two of these established populations found on Lonquimay was just 1.54% ($N = 2$, populations 7–8, 95% CI −1.82 to +2.02%), attributing the majority of differentiation in the established group to the two more distant southern populations. Variance among populations colonising Lonquimay was 7.02% ($N = 5$, populations 1–5, 95% CI 4.84–9.29%).

Bayesian analysis corroborates the trend from AMOVA in F_{ST} values (Fig. 3) both overall and for each population group. The lowest DIC value for Bayesian analysis of genetic variance among populations was obtained for the full model (DIC = 5,424). The full model's estimated value of theta-II was 6.33% [$N = 10$, 95% credible interval (Cr.I.) 5.43–7.32%]. For established populations, theta-II was 9.43% ($N = 4$, populations 7–10, 95% Cr.I. 6.70–11.87%), 0.42% ($N = 2$, populations 7–8, 95% Cr.I. 7.55×10^{-5} to 1.59%) for the two of those established populations on Lonquimay and 4.96% for colonising populations ($N = 5$, populations 1–5, 95% Cr.I. 2.88–7.00%).

Bayesian analysis of the structure of these populations produced only one optimal partition (log likelihood −9,614.63), separating population 9 from all others. One Lonquimay individual from population 3 was assigned to this separate group (Online Resource 2). Admixture analysis showed no individuals to have mixed genetic heritage. SplitsTree NeighborNet analysis of AFLP data also revealed southern populations (9–10) to be genetically distanced from Lonquimay populations (Online Resource 3).

Discussion

Fast recovery from weak founder effect

For determination of founder effects we here rely on metrics of genetic diversity within colonising populations in relation to established populations nearby. Genetic variation and divergence metrics from AFLPs are generally higher in older populations of *N. argentea* (Table 2; Fig. 2), but the differences between categories are not statistically significant. A moderate reduction suggests that there might have been founder effects, but levels of within-populational variation are very similar in colonising and established populations. This study joins a body of literature that displays negative results toward founder effect presence.

Reductions of private bands and rarity index in colonising populations indicate that a founder effect was once present in colonising populations. The high genetic similarity and likely gene flow between populations on the volcano points to genetic rescue (Ingvarsson 2001) from incoming propagules to enhance the variety of bands present and counteract any losses due to genetic drift. Some genetic links to populations further afield is likely (population 9, Online Resource 2), with one colonising individual probably coming from the Chilean-Argentinean border. This assignment could be attributed to bird-mediated dispersal, which has been observed before (Castor 2002). The assignment of all Lonquimay populations to a single cluster points to intense and ongoing genetic mixing between populations on this volcano and greater affinities to populations close by Volcán Llaima. In other words, gene flow seems to be sufficient such that all individuals of the Lonquimay and Llaima region behave as a single

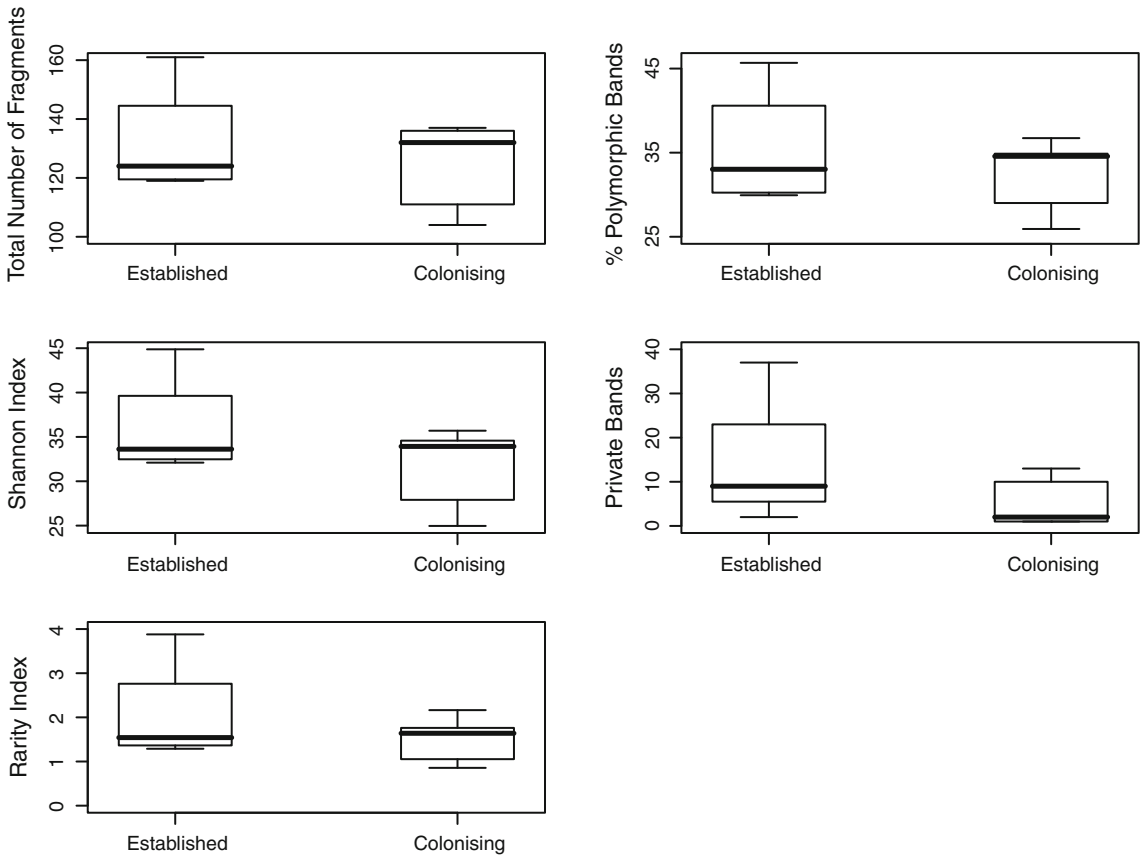

Fig. 2 Box plots of genetic diversity and divergence metrics for established (7–10) and colonising populations (1–5) of *Nassauvia argentea*, revealing a slight decrease in variation and divergence in recently colonised populations. The box is between upper and lower quartiles, the thick line in the middle of the box is the median and the whiskers

population. This finding parallels results for *Nassauvia lagascae* and *Hypochaeris tenuifolia*, where populations of the Lonquimay, Llaima and Paso Pino Hachado region are also assigned to the same group by Bayesian analysis (López et al. 2010; Tremetsberger et al. 2003 respectively). High levels of intermixing have also been found in other studies of the genetics of volcano colonisation (Yang et al. 2008). This genetic rescue would have taken place between 1990 and 2003, i.e. in not more than 14 years, making the founder effect only weakly discernible.

This speed of recovery of genetic diversity in *N. argentea* concurs with other molecular studies of colonisation on continental volcanoes. Findings from del Moral and Clampitt (1985) on Mount St. Helens implied that colonising population dynamics occur soon after eruptions on continental volcanoes. Yang et al. (2008) confirmed this with molecular data, with no founder effect being present 24 years after colonisation of Mount St. Helens. To this we can add the absence of any strong founder effects in *N. lagascae* (López et al. 2010) and *H. tenuifolia* (Tremetsberger et al. 2003) based on AFLPs from Lonquimay

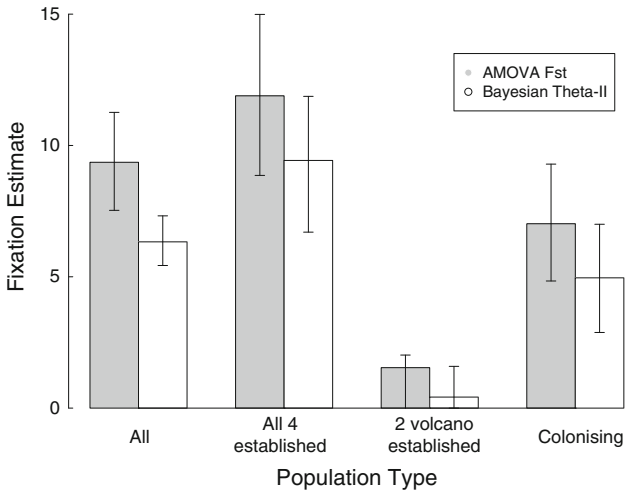

Fig. 3 Bar chart contrasting F_{ST} estimates from AMOVA and Bayesian analyses of population differentiation among all ten populations, all established populations (7–10), established populations on Lonquimay (7–8) and colonising populations (1–5) of *Nassauvia argentea*. Error bars represent 95% confidence interval for AMOVA and 95% credible interval for Bayesian analysis

Table 3 Comparison of established population characteristics of *Nassauvia lagascae* var. *lanata* (data from López et al. 2010; populations designated by collection numbers) and *N. argentea*

	Population size	Area (m^2)	Mean plant diameter	Mean plant height (cm)	Mean number of shoots	Number of flowering shoots per reproductive individual	Mean herb layer height (cm)
N. argentea							
7	1,500	30,000	8.0	7.5	23.76	3.50	6.93
8	30,000	600,000	20.0	10.0	108.80	14.88	11.86
9	X	X	8.0	X	14.20	1.33	4.00
10	X	X	24.0	5.3	311.83	13.10	10.00
Mean	15,750	315,000	15.0	7.6	114.6	8.2	8.2
SD	20,153	403,051	8.2	2.4	138.2	6.8	3.5
N. lagascae							
3	50	1,000	X	X	10.0	X	3.0
4	3,000	6,000	3	1.0	8.0	3	4.4
5	100	5,000	7	1.8	14.5	1	1.9
6	500	10,000	5	2.0	8.5	3	6.5
6A	1,000	3,000	9	2.5	23.0	3	10.5
7	1,000	30,000	6	3.0	10.0	3	4.0
14	300	7,500	6	3.0	17.0	3	5.1
Mean	850	8,929	6.0	2.2	13.0	2.6	5.1
SD	1,024	9,740	2.0	0.8	5.5	0.8	2.8

X missing data

12–14 years after the most recent eruption, though hints of past founder effects were observed in population differentiation.

A high among-population divergence value in colonising populations is consistent with a small number of founders from multiple source populations (Wade and McCauley 1988). A wide source area of propagules, including populations not sampled here, may have contributed to the higher F_{ST} among colonising populations compared to the two populations established on Lonquimay (Fig. 3). As the established Lonquimay populations have aged they may have undergone a succession-like genetic process whereby the genetic makeup changes as some bands are fixed and others lost, contributing to a fall in F_{ST}. A snapshot of this change can be seen in the intermediate genetic diversity values of the surviving population (Table 2), which contained individuals colonising the ash and also surviving beyond it. The process of genetic recovery with greater population divergence appears to be ongoing.

Genetic diversity in relation to population characteristics

Demographic characteristics did not account for levels of genetic diversity in individual *N. argentea* populations, as would be consistent with founder effect theory. Rather, it may be the characteristics of the species itself that can determine whether or not founder effects are observed. With 500–5,000 plants (median = 2,000), colonising populations are large, though not yet comparable to some established populations (e.g., an estimated 30,000 plants in population 7; Online Resource 1). Similarly, the area occupied by populations is generally large (though again lower for colonising populations). Compared with other pioneers on volcanoes, *N. argentea* is vigorous with at least 33% of individuals in colonising populations being reproductive 14 years after the eruption, compared to no colonisers of *V. membranaceum* (black huckleberry) reproducing on Mount St. Helens 24 years after the last eruption (Yang et al. 2008). This relative vigour of colonising populations of *N. argentea* could explain fast genetic rescue of genetic diversity in its colonising populations.

Weaker founder effects in *N. argentea* than in *N. lagascae*

The close relatives *N. argentea* and *N. lagascae* are both pioneer species of the volcanic ashfields emanating from the 1988 Navidad eruption. When we compare the results that colonisation had on genetic diversity measures in the two species, we can see evidence for a weaker founder effect or faster recovery in *N. argentea* than in *N. lagascae* (López et al. 2010). In the latter species, the colonising populations have significantly lower divergence metrics (in terms of rare and private AFLP bands) and insignificantly lower levels of within-populational genetic variation.

Differences in genetic diversity in the same disturbed environment are likely caused by differences in population growth and immigration in the 14 years since the vacant habitat was formed. Species differences in the nearly full genetic restoration of *N. argentea*, in contrast to *N. lagascae*, within 14 years could be attributed to differences in species characteristics. *N. argentea* are larger plants in terms of diameter, height and number of shoots of plants, with larger population size and coverage than *N. lagascae* (Table 3). These features may be important in enabling the genetic diversity of a species to recover faster. Interestingly, both *Nassauvia* species lack a specialised syndrome that would allow long-distance dispersal (the achenes of both species have a smooth surface and deciduous pappus; Castor 2002), though secondary dispersal of propagules by small animals could be involved.

Our study also provides recommendations for the use of automatic scoring of AFLPs. We suggest that more appropriate parameter choices can be made if a wider range of parameter settings is tested than those included in Holland et al. (2008) or Arrigo et al. (2009), while still following their broad methodology. More certainty can also be obtained if a combination of optimisation metrics is considered when making decisions to obtain suitable information content while also minimising the mismatch error rate. This method is definitely worth the effort given the ability of automatic scoring software to score all samples in this study in 35 s and the reproducibility of results under certain settings deepens one's understanding of the patterns of variation observed.

In conclusion we found insufficient support for a founder effect in genetic diversity of *N. argentea* populations colonising Lonquimay 14 years after the catastrophic Navidad eruption. This is most likely due to strong gene flow from established populations. Levels of gene flow probably exceed those seen in a less vigorous sister species where a slightly stronger founder effect was found. Longitudinal studies of colonisation tracking the time of arrival in relation to their dispersal and reproductive properties of species would be a useful empirical test of the genetic rescue hypothesis with respect to founder effect theory.

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