Bottleneck effects in local populations of fossorial *Ctenomys* (Rodentia, Ctenomyidae) affected by vulcanism

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Bottleneck effects in three local populations of the fossorial rodent *Ctenomys maulinus brunneus* as caused by the recent eruption of the Lonquimay volcano in the Andes of Southcentral Chile are presented. Comparative census estimates in Río Colorado indicated a 91.3 per cent decrease in the breeding population size after the catastrophe. All parameters of genetic diversity were drastically affected and surpassed neutral expectations in each population. The proportion of polymorphic loci decreased by 57 per cent, 100 per cent and 83.2 per cent in the bottleneck populations of Río Colorado, Las Raíces and Alto Bío Bío, respectively. The source populations were estimated to have 2.2, 1.5 and 1.4 alleles per locus whereas the three derived populations had estimated values of 1.4, 1.0 and 1.1, respectively. Average heterozygosity dropped by 71 per cent, 100 per cent, and 57 per cent in the same populations, respectively. The spatial genetic structuring observed before the eruption indicated a high degree of population subdivision (Wahlund effect), consistent with an isolation-by-distance model. After the eruption, excessive microspatial genetic differentiation and larger-scale homogeneity indicated drastic disruption of the breeding or social units. Low levels of genetic variation in Andean *Ctenomys*, claimed to be an adaptive response to the stable subterranean niche, can be attained by the recurrent catastrophe-induced effects of genetic drift.

Keywords: allozymic variation, bottlenecks, *Ctenomys*, genetic drift, natural catastrophes, vulcanism.

Introduction

Natural catastrophes affecting wild animal populations in fragmented habitats have significant evolutionary repercussions (McCauley, 1991) because the unpredictable nature of demographic declines makes it necessary to evaluate their implications for population dynamics (Chepko-Sade *et al.*, 1987) and genetics (Maruyama & Fuerst, 1985a,b). Demographic bottlenecks associated with repeated catastrophes are central to issues on biological conservation (Lande & Barrowclough, 1987; Lande, 1988) and to speciation events promoted by founder effects (Barton, 1989; Rice & Hostert, 1993). Because of the random loss of alleles following a bottleneck, there will be a direct decrease in heterozygosity according to its severity (Nei *et al.*, 1975; Chakraborty & Nei, 1977). Further bottleneck effects involve substantial changes in the spatial genetic structure of animal populations of restricted vagility (Johnson, 1988; Coates, 1992), or induce new selective trends by changing the internal genetic environment

(Bryant & Meffert, 1990; McCommas & Bryant, 1990).

Owing to the lack of pre-existing suitable genetic and demographic data, paradigmatic reports of present-day genetic homogeneity in mammals have used those levels to imply putative bottlenecks of varied origins (Bonnell & Selander, 1974; O'Brien *et al.*, 1985, 1987; Harwood & Hall, 1990). In this context, natural disasters are a source of demographic bottlenecks linked to environmental stochasticity (Wade & McCauley, 1988). They affect demic persistence by reducing the population size (Lande, 1988; McCauley, 1991) and thus are instrumental for the random effects of genetic drift (Easteal, 1985; Leberg, 1992).

The natural catastrophe exemplified by the recent eruption of the Lonquimay volcano in the Andes of Southcentral Chile (Moreno & Gardeweg, 1989; Barrientos & Acevedo-Aranguiz, 1992) provided a natural scenario to study bottleneck effects on the subterranean rodent *Ctenomys maulinus brunneus*, monitored before and after the eruption. Because

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theory predicts a direct relation between the genetic losses and the severity of a demographic crash (Chakraborty & Nei, 1977), we aim to find such an empirical corroboration.

Materials and methods

Field studies were conducted in the austral spring and summer 1986–87 and 1991, in two populations of *Ctenomys maulinus brunneus* ($2n=26$) and in one population of a parapatric 28-chromosome cytotype (Gallardo & Köhler, 1992). The localities, geographical coordinates and numbers of specimens per population examined before the eruption are as follows: *C. m. brunneus* ($2n=26$): Río Colorado ($38^{\circ}25'S$, $71^{\circ}32'W$), 51; Las Raíces ($38^{\circ}25'S$, $71^{\circ}27'W$), 9. *Ctenomys* sp. ($2n=28$): Alto Bío Bío ($38^{\circ}39'S$, $70^{\circ}25'W$), 19 (Fig. 1). Río Colorado is 4 km² of Andean steppe at 1450 m altitude corresponding to the *Festucetea thermari* phytosociological formation (Freiberg, 1985). The vegetation consisted of a 100–300 m belt of bunchgrass (*Anthoxanthum juncifolium*, *Festuca thermarum* and *Stipa* sp.), replaced altitudinally by herbaceous annuals (*Adesmia emarginata*, *Baccharis nivalis* and *Pernetia pumila*). Direct pre-eruption demographic estimates in Río Colorado were obtained by the total removal of animals trapped within four grids differing in size, vegetation type and plant coverage at places showing recent digging activity. Site A: one 50 × 70 m grid on consolidated friable soils covered by 80–90 per cent of bunchgrass, interspersed with annual plants. Site B: two grids (40 × 60 m and 50 × 50 m) on loose volcanic sediments covered by 40–50 per cent of annuals. Site C: a single 60 × 140 m grid on loose volcanic soils with 20–25 per cent coverage of annual plants. The inactivity of the Lonquimay volcano was punctuated in December 1988 by a violent 6-month eruptive phase (Moreno & Gardeweg, 1989; Barrientos & Acevedo-Aranguiz, 1992) that totally destroyed

the annual vegetation and concomitantly reduced the population density of herbivorous *Ctenomys*. No animal activity was observed after the eruption, except in site A where a one hectare grid was sampled during the summer of 1991. No similar census data exist for the populations of Las Raíces and Alto Bío Bío.

Pre-eruption estimates of electrophoretic variation were retrieved from previously published results of proteins encoded by 23 loci surveyed in Río Colorado (Gallardo & Palma, 1992), 18 in Las Raíces and 19 in Alto Bío Bío (Gallardo & Köhler, 1992). The loci assayed were: isocitrate dehydrogenase (*ICD-1*, *ICD-2*, Enzyme Commission No. 1.1.1.42), malate dehydrogenase (*MDH-1*, *MDH-2*, E.C. 1.1.1.37), glutamate-oxaloacetate transaminase (*GOT-1*, *GOT-2*, 2.6.1.1), glycerol-3-phosphate dehydrogenase (*GPD*, 1.1.1.8), glucose 6-phosphate dehydrogenase (*Gd*, 1.1.1.49), glucose dehydrogenase (*GDH*, 1.1.1.47), lactate dehydrogenase (*LDH-1*, *LDH-2*, 1.1.1.27), xanthine dehydrogenase (*XDH*, 2.7.1.1), phosphoglucumutase (*PGM-2*, *PGM-3*, 2.7.5.1), glucose phosphate isomerase (*GPI-1*, 5.3.1.9), phosphogluconate dehydrogenase (*PGD*, 1.1.1.44), albumin (*ALB*), transferrin (*TRFER*), hexokinase (*HK*, 2.7.1.1), acid phosphatase (*ACP-1*, *ACP-2*, 3.1.3.2) and malic enzyme (*ME*, 1.1.1.40). Fifty-eight animals from Río Colorado, 23 from Las Raíces and 18 from Alto Bío Bío were live-trapped and screened for the same loci after the eruption. Tissue homogenates, buffer systems, migration conditions and mixtures were prepared according to the methods of Selander *et al.* (1971). Heterogeneity of allele frequencies among the populations was investigated for each locus with contingency χ^2 analysis using the method of Workman & Niswander (1970).

Taking into account the univoltine condition and the species' unbiased sex ratio (Gallardo & Anrique, 1991), demographic changes of the breeding population size in Río Colorado were evaluated before and after the eruption by the formula $N_e = 4N_m \times N_f /$

Fig. 1 Map of the study area showing the three collecting localities of *Ctenomys maulinus brunneus* in the Andes of Southcentral Chile.

($N_m + N_f$) where N_m and N_f represent the numbers of males and females, respectively (Hartl & Clark, 1989). This estimate provided a rough approximation below which N_e decreases depending on departures from idealized structure (Husband & Barrett, 1992).

An indirect method assuming neutrality in finite populations was used to estimate the N_e of our group samples, independent of census data (Johnson, 1988). This approach, using the fixation index between populations separated by the eruption, is given by the formula: $F_{ST} = 1 - [1 - (1/2N_e)]^t$. Estimations of N_e were based on $t = 3$ generations separating the source from the bottleneck populations, according to the univoltine reproductive attribute of the species (Gallardo & Anrique, 1991).

A statistical comparison of the fit of the observed genotypic ratios to Hardy-Weinberg expectations was made on a locus-by-locus basis for each population, using a contingency table (Swofford & Selander, 1989). The expected loss of heterozygosity under neutral conditions, assuming a rapid rate of return to equilibrium after the eruption, was estimated as $H_t = H_0[1 - (1/2N_e)]^t$, where N_e is the effective population size over the time elapsed ($t = 3$) and H_0 is the initial heterozygosity value, based on Hardy-Weinberg expectations (Easteal, 1985; Leberg, 1992). This estimate of mean heterozygosity is considered a better measure of genetic variation than direct count heterozygosity (Nei & Roychoudhury, 1974). Expected and observed heterozygosity values were compared and tested for significance by the nonparametric Wilcoxon matched-pairs signed-ranks test (Leberg, 1992), and by a paired t -test of arcsin transformed genetic data using all loci examined (Archie, 1985). As the results of both types of tests were identical, only the results of the parametric test will be presented.

Wright's two-level hierarchical analyses of breeding structure (Wright, 1965) for subdivided populations were used to identify sources of spatial differentiation in gene frequencies before and after the eruption of Río Colorado. After negative testing for sex-linkage, the first hierarchical level consisted of the within-grid animals whereas the second level was formed by the animals sampled outside the grid. All computations of genetic variation were performed with BIOSYS-1 (Swofford & Selander, 1989).

Results

There was a direct correlation between plant coverage and animal density before the eruption in Río Colorado. The 23 animals captured in vegetation-rich site A gave an estimate of 66 animals/ha. Pooled density estimates from both grids in semi-vegetated site

B indicated 35 animals/ha whereas only 8.3 animals/ha were estimated in the scarcely vegetated site C. Similar estimates (3–47 animals/ha to 218 animals/ha) have been found in other *Ctenomys* species (Pearson, 1959; Pearson *et al.*, 1968).

By using the correlation between plant resources and animal abundance, direct estimates of the breeding population size indicated 1930 animals before the eruption. As 20 animals (10 males, 10 females) were removed from the one-hectare site A after the eruption, its extrapolation indicated only 168 animals, which corresponds to a 91.3 per cent decrease of population size.

The standardized variance of gene frequency ($F_{ST} = 0.174$) indicated that about 83 per cent of the total variance in Río Colorado was from intrapopulational differentiation after the eruption. Larger intrapopulational variances were observed in Las Raíces ($F_{ST} = 0.318$) and in Alto Bío Bío ($F_{ST} = 0.321$). Estimates of N_e based on these indices ranged from four individuals (Las Raíces and Alto Bío Bío) to eight individuals in Río Colorado. This result suggests that under neutrality, a fixation index 20–36 times smaller than the observed values would match the total number of pre-eruption reproductive adults as indicated by census data. If the breeding size ($N = 168$) is maintained, the changes observed are comparable to neutral effects prolonged by 65 generations (Río Colorado) to 130 generations in the other two populations.

The bottleneck has left a clear imprint on the genetic make-up of these populations. Eight previously polymorphic loci in Río Colorado, all in Las Raíces, and five in Alto Bío Bío became monomorphic after the eruption (Table 1). There was a general tendency for the loss of rare alleles as predicted by general bottleneck effects. Nevertheless, the reverse holds for the common alleles of loci *PGD* and *PGM-2* in Las Raíces, and loci *Gd* and *GDH* in Alto Bío Bío. The drastic reduction in frequency or the loss of these high frequency alleles reflects the pervasive effects of genetic drift. The average observed heterozygosity dropped from 3.1 per cent to 0.9 per cent in Río Colorado and from 4.2 per cent to 1.8 per cent in Alto Bío Bío, and decreased to its most extreme minimal value (from 4.3 per cent to 0 per cent) in Las Raíces (Table 1). Similarly, the number of polymorphic loci (0.95 per cent) dropped from 30.4 per cent to 13 per cent in Río Colorado and from 31.6 per cent to 5.3 per cent in Alto Bío Bío. The most dramatic change (from 50 per cent to 0 per cent) was observed in the Las Raíces sample. Contingency χ^2 analyses conducted before and after the bottleneck revealed significant heterogeneity in allele frequencies among populations (Río Colorado, $\chi^2_{14} = 203.89$, $P = 0.0000$; Las Raíces, $\chi^2_5 = 116.23$, $P < 0.0000$; Alto Bío Bío, $\chi^2_8 = 104.24$, $P < 0.0000$).

Table 1 Changes in allele frequencies at polymorphic loci in three populations of *Ctenomys maulinus brunneus* affected by the eruption of volcano Lonquimay, Malleco province, Chile

Locus	Río Colorado		Las Raíces		Alto Bío Bío	
	Pre N= 51	Post N= 58	Pre N= 9	Post N= 23	Pre N= 19	Post N= 18
<i>ICD-1</i>						
<i>A</i>	0.020	0.000			0.000	0.000
<i>B</i>	0.980	1.000			0.947*	1.000
<i>C</i>	0.000	0.000			0.053	0.000
<i>MDH-1</i>						
<i>A</i>	0.971	1.000	0.944	1.000		
<i>B</i>	0.029	0.000	0.056	0.000		
<i>GOT-1</i>						
<i>A</i>	0.010	0.000				
<i>B</i>	0.990	1.000				
<i>GPD</i>						
<i>A</i>	0.961*	1.000				
<i>B</i>	0.039	0.000				
<i>Gd</i>						
<i>A</i>			0.000*	0.000	0.158*	0.833
<i>B</i>			0.778	1.000	0.842	0.167
<i>C</i>			0.222	0.000	0.000	0.000
<i>GDH</i>						
<i>A</i>			0.889	1.000	0.395*	1.000
<i>B</i>			0.111	0.000	0.553	0.000
<i>C</i>			0.000	0.000	0.053	0.000
<i>LDH-2</i>						
<i>A</i>	0.843*	1.000	0.889	1.000		
<i>B</i>	0.157	0.000	0.111	0.000		
<i>XDH-1</i>						
<i>A</i>	0.157*	0.034*	0.778*	1.000	0.000*	0.000
<i>B</i>	0.647	0.026	0.000	0.000	0.158	0.000
<i>C</i>	0.196	0.940	0.222	0.000	0.842	1.000
<i>PGM-2</i>						
<i>A</i>	0.814	1.000	0.444	1.000	0.289*	0.000
<i>B</i>	0.186	0.000	0.556	0.000	0.447	1.000
<i>C</i>	0.000	0.000	0.000	0.000	0.263	0.000
<i>PGM-3</i>						
<i>A</i>	0.059	0.000	0.000	0.000		
<i>B</i>	0.941	1.000	0.944	1.000		
<i>C</i>	0.000	0.000	0.056	0.000		
<i>GPI-1</i>						
<i>A</i>	0.951*	1.000	0.000	0.000		
<i>B</i>	0.049	0.000	0.944	1.000		
<i>C</i>	0.000	0.000	0.056	0.000		
<i>PGD</i>						
<i>A</i>	0.078*	0.181*	0.778*	0.000	0.000	0.000
<i>B</i>	0.902	0.793	0.222	1.000	0.079	0.000
<i>C</i>	0.020	0.026	0.000	0.000	0.921	1.000
<i>HK</i>						
<i>A</i>	0.098*	0.078				
<i>B</i>	0.902	0.922				
<i>ACP-2</i>						
<i>A</i>	0.098*	0.026				
<i>B</i>	0.902	0.974				
Polymorphism (%)	30.4	13.0	50.0	0.0	31.6	5.3
Alleles/locus	2.2	1.4	1.5	1.0	1.4	1.1
Heterozygosity direct count (%)	3.1	0.9	4.3	0.0	4.2	1.8

*Genotypes frequencies for these loci and populations deviate significantly ($P < 0.01$) from Hardy–Weinberg expectations.

The proportional final losses in heterozygosity for the three populations in relation to their initial values following neutral expectations are given in Table 2. Significant differences, exceeding a neutral model for equality between initial and final mean heterozygosity, were detected by the results of the *t*-tests and the Wilcoxon tests (Table 2). The nonsignificant result of this test in Alto Bío Bío ($P = 0.11$) is probably based on chance alone. The results of 27 pre-eruption tests for fit to Hardy–Weinberg expectations indicated that 57 per cent of all comparisons differed significantly from the expected distribution at the 0.05 or lower level. Ignoring statistical significance, 79 per cent of the total post-eruption comparisons showed heterozygote deficiency, and 21 per cent a heterozygote excess. The

only polymorphic locus in Alto Bío Bío showed an excess of heterozygosity after the eruption whereas a marked deficit was apparent in two of the four polymorphic loci of Río Colorado. Thus, there are consistent deviations from Hardy–Weinberg in our data set that suggest pervasive substructuring within our sample units.

Multilocus measures of genetic differentiation among pairs of populations gave heterogeneous results. The pre-eruption mean genetic similarity (Rogers, 1972) between Río Colorado and Las Raíces ($S = 81.7$ per cent) increased to 84.2 per cent after the eruption. However, the similarities between Las Raíces–Alto Bío Bío (52 per cent) and Río Colorado–Alto Bío Bío (57.8 per cent) dropped to 45 per cent and 50.1 per

Table 2 Observed pre-bottleneck heterozygosities (H-Pre), expected heterozygosities under neutral expectations (H-Exp) and observed post-bottleneck heterozygosities (H-Post) of polymorphic loci from three populations of *Ctenomys maulinus brunneus* affected by a bottleneck

No. of loci Locus	Population								
	Río Colorado			Las Raíces			Alto Bío Bío		
	23			18			19		
	H-Pre	H-Exp	H-Post	H-Pre	H-Exp	H-Post	H-Pre	H-Exp	H-post
<i>ICD-1</i>	0.039	0.026	0.000				0.102	0.068	0.000
<i>MDH-1</i>	0.058	0.039	0.000	0.111	0.074	0.000			
<i>GOT-1</i>	0.020	0.013	0.000						
<i>GPD</i>	0.076	0.051	0.000						
<i>Gd</i>				0.366	0.245	0.000	0.273	0.183	0.286
<i>GDH</i>				0.209	0.140	0.000	0.550	0.368	0.000
<i>LDH-2</i>	0.267	0.179	0.000	0.209	0.140	0.000			
<i>XDH-1</i>	0.523	0.350	0.116	0.366	0.245	0.000	0.273	0.183	0.000
<i>PGM-2</i>	0.306	0.205	0.000	0.523	0.350	0.000	0.664	0.445	0.000
<i>PGM-3</i>	0.112	0.075	0.000	0.111	0.074	0.000			
<i>GPI-1</i>	0.094	0.063	0.000	0.111	0.074	0.000			
<i>PGD</i>	0.182	0.122	0.340	0.366	0.245	0.000	0.149	0.100	0.000
<i>HK</i>	0.179	0.120	0.144						
<i>ACP-2</i>	0.179	0.120	0.051						
Mean	0.088	0.059	0.028	0.132	0.063	0.000	0.106	0.071	0.015
Parametric comparisons									
Pre vs. Exp									
Paired <i>t</i> -test		3.95			3.68			2.52	
Probability		0.001			0.002			0.021	
Pre vs. Post									
Paired <i>t</i> -test		3.28			3.78			2.28	
Probability		0.003			0.001			0.035	
Exp vs. Post									
Paired <i>t</i> -test		-2.77			-3.80			-2.16	
Probability		0.011			0.001			0.045	

cent after the eruption, respectively. Nevertheless, the overall degree of genetic relatedness between the samples was the same before and after the eruption.

The overall bottleneck consequences affecting the degree of interpopulational differentiation were estimated by the standardized variance of gene frequencies (F_{ST}) computed between pairs of population samples. This pre-eruption index (F_{ST}) for the pairs formed by the Río Colorado and Las Raíces samples increased from 0.336 to 0.881 after the eruption. Similarly, the differentiation between the pair Río Colorado–Alto Bío Bío increased from 0.638 to 0.908, whereas the pair formed by Las Raíces–Alto Bío Bío was raised from 0.633 to 0.969. By inspecting the pattern of microspatial genetic differentiation of the Río Colorado population, the within-grid fixation index ($F_{ST}=0.020$) corroborated a negligible spatial genetic differentiation between the sexes as is expected in a panmictic unit. The heterogeneity test for differences in the within-grid gene frequencies was nonsignificant ($P<0.576$). Further agreement was found by the highly significant difference between the grid relative to the animals from outside the grid ($P<0.0001$) and the fourfold increase in the corresponding fixation index ($F_{ST}=0.078$). This pattern of pre-eruption population structuring supports the proposition of isolate breaking (Wahlund effect) where random mating and rates of gene exchange are governed by the size of the breeding or social units. The pattern uncovered was reversed after the eruption, indicating an excess of microgeographical differentiation but larger-scale homogeneity. The within-grid fixation index increased by an order of magnitude ($F_{ST}=0.24$) and significant microspatial differences between sexes were obtained ($P=0.0022$). This level of microdifferentiation contrasted with the homogeneity inferred from the nonsignificant differences between the grid relative to the remaining sampled animals ($P<0.708$; $F_{ST}=0.009$).

Discussion

Much information dealing with karyotypical (Gallardo, 1979, 1991), parasitological (Beaucournu & Gallardo, 1977), ecological (Gallardo & Anrique, 1991) and allozymic data (Gallardo & Köhler, 1992; Gallardo & Palma, 1992) of this species has allowed the appreciation of its evolutionary dynamics as a result of 20 years of field observations.

Although no previous estimates of the breeding population size have been reported for *Ctenomys*, our census data fall within the range specified for other congeneric species (Pearson, 1959; Pearson *et al.*, 1968). Present estimates of N_e based on the temporal variations in allele frequencies (four to eight animals)

seem to be very small compared with the census size of the Río Colorado population. Although substantial levels of population substructuring may prevail, it is difficult to match the results of the indirect estimate with pre-existing life history data of the species (Gallardo & Anrique, 1991) or to envisage an idealized, panmictic population of two males and two females. This discrepancy may reflect the extreme sensitivity of the method to the number of generations used, restricted participation of the adults in mating, or lack of representativeness of the genotype frequencies in a local sampling (Nei & Tajima, 1981). Besides, they apply strictly to the founder generation and thus do not include the cumulative effects of post-founding generations (Easteal, 1985). This ignores the effect of the intrinsic growth rate and maximizes the loss of genetic variability by drift (Baker & Moeed, 1987). In this respect, the same level of genetic effects to the observed values reported here would require a fixation index considerably smaller or a number of generations greatly exceeding the actual data. Underestimations about one order of magnitude relative to census data have also been found using the same method in plants (Husband & Barrett, 1992; Eguiarte *et al.*, 1993).

Indirect estimates of N_e in small mammals, based on the mutation rate of non-differentially staining chromosomes, range from 30 to 200 individuals per population (Lande, 1988). Nevertheless, standard staining methods underestimate chromosomal repatterning by a factor greater than 4.5 (Haiduk *et al.*, 1981) and thus are not robust estimates of N_e in small mammals.

Effective population size, being less than the actual number of breeding adults, can also be reduced by demographic fluctuations over generations (Randi & Apollonio, 1988). In fact, the average N_e over generations fluctuating in size is not the arithmetical but the harmonic mean of the N_e over time (Maruyama & Kimura, 1980). The Lonquimay volcano has previously erupted in 1853, 1887 and 1940 (Moreno & Gardeweg, 1989). Provided that there were similar demographic effects, the genetic pool of adjacent populations may have been disrupted in generations 135, 101 and 48 before the present. Besides, as the nearby volcanoes Antuco, Llaima and Villarrica have had a total of 36 eruptive events since 1640 (Veblen, 1985), the long-term effective population size in Andean *Ctenomys* populations is much smaller than any single estimate.

A substantial and prolonged reduction of population size is commonly expected to result in a loss of genetic diversity, reduced heterozygosity and increased differentiation between populations (Chakraborty & Nei, 1977; McCommas & Bryant, 1990). Although fewer

than 10 founders are predicted to diminish substantially heterozygosity levels and the number of alleles per locus, significant effects were observed with larger samples. The group of high frequency alleles was retained in the three populations after the bottleneck, irrespective of sample size, whereas the generalized loss of rare alleles did lower the average heterozygosity values of the sampled groups.

Loss of heterozygosity, significantly exceeding neutral expectations in these populations, could be attributed to slow recovery after the bottleneck (Baker & Moed, 1987). In fact, *Ctenomys* has a low intrinsic growth rate (Reig *et al.*, 1990). However, given the recurrence of natural catastrophes, the recovery rate is not the only reason for the loss of heterozygosity in these populations. In this respect, note that the effects of inbreeding on heterozygosity levels do not necessarily come from consanguineous matings because low rates of dispersal and limited N_e can also produce a generalized form of inbreeding affecting heterozygosity values (Chesser & Ryman, 1986). Accordingly, restricted mobility and territorial behaviour in *Ctenomys* allow gene exchange with a relatively small number of individuals regardless of whether they are distributed continuously or in discrete patches, thus conforming to an isolation-by-distance model (Slatkin, 1993). Regardless of the actual combination of founder and post-founder effects, the implication of low allozymic variation is that random sampling of alleles, breeding structure and low dispersal capabilities in fragmented habitats have amplified the effects of genetic heterogeneity produced by the eruption.

The pervasive effects of the bottleneck are also reflected by shifts in the breeding structure of the Río Colorado population. The pre-eruption hierarchical tests suggested that a Wahlund effect underlay our nested comparisons. Nevertheless, the post-eruption *F*-statistics indicated an excess of microgeographical differentiation and larger-scale homogeneity. This pattern is attributed to disruption of the microspatial breeding or social units whereas random fluctuations in a depleted genetic pool cancel out one another at a larger spatial scale.

The importance of bottlenecks in inducing speciation by genetic revolution has been summarized by Carson & Templeton (1984). A common experimental design has used repeated bottleneck/exponential-growth cycles in *Drosophila*, subsequently assayed for pre- and post-zygotic reproductive isolation (Dodd & Powell, 1985). The similar protocol used by Meffert & Bryant (1991) also demonstrated some changes in the components of mating behaviour, but none leading to pre-zygotic isolation. Even multiple bottlenecks have led to weak levels of pre- and post-zygotic isolation (Rice & Hostert, 1993).

Repeated demographic crises are also pivotal to species vulnerability (O'Brien & Everman, 1988) and thus are central to biological conservation (Berry, 1986). Populations reaching critical demographic values because of bottleneck cycles should be at risk through inbreeding depression (O'Brien *et al.*, 1985; Wildt *et al.*, 1987) or through evoking a similar immunological response to environmental disturbances (O'Brien *et al.*, 1985). Population persistence can also be affected by loss of adaptive genetic variability, low fecundity rates (Packer *et al.*, 1991) or reduced mating success derived from critical population size (Lande, 1987).

Physiographical and vegetational discontinuities in the Andes (Simpson, 1979; Veblen, 1985) are formidable spatial barriers to interdemographic gene flow in fossorial *Ctenomys* (Gallardo & Köhler, 1992). Post-eruption observations indicated that animals distributed in ecotonal areas close to the forests survived the deleterious precipitation of ashes and scoria deposition. Peripheral animals were protected by the shield effect of trees and now are contributing as founders to the recolonization of vacant areas. Several demographic crises, resulting from repeated near-to-extinction and colonization cycles during Andean *Ctenomys* lineage evolution, could have preceeded and possibly driven *C. m. brunneus* speciation, resulting in karyotypically uniform populations with very low genetic variability (Gallardo & Köhler, 1994). Accordingly, long-term erosion of genetic variation mimics a regime of stabilizing selection operating in the physically stable subterranean environment (Nevo, 1979, 1990) as exemplified by the low biochemical variability in geographical isolates of the subterranean rodent *Spalacopus cyanus* (Gallardo *et al.*, 1992). Random genetic losses caused by recurrent natural disasters produce the same mosaic pattern of low interpopulational allozymic variation in *Ctenomys*, and its decline is mainly derived from the severity of historical chance events (Gallardo & Köhler, 1992). In view of the active orogenetic process in the Andes, each *Ctenomys* population represents a transient dynamic system fluctuating between near-to-extinction and recolonization (Hanski, 1991; Hanski & Gilpin, 1991). Thus, a demographic explanation, together with the evaluation of the time since the last bottleneck, must be considered when analysing populations recovering from near-to-zero variability (Maruyama & Fuerst, 1985a,b).

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