Genetic variation within and between four chromosomal races of *Liolaemus monticola* in Chile

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Allozyme variability was assessed within and between18 samples of four chromosomal races of the *Liolaemus monticola* complex: "Southern, 2n=34", "Northern, 2n=38–40", "Multiple Fission, 2n=42–44" and "Northern modified 1, 2n=38–40". This is an endemic montane Chilean lizard characterized by extensive chromosomal polytypy. The population genetic structure was studied by means of allozyme electrophoresis of 20 presumptive loci. Population heterogeneity analysis carried out by the estimation of Weir and Cockerham's *F*-statistic (θ), demonstrated substantial genetic differentiation among populations. The u-statistic, genetic distance data and multivariate analyses show that genetic variation is distributed into geographically coherent population groups in accordance with three of the four chromosome races. The greatest differentiation occurs between all populations of the "Southern, 2n=34" race and a second group that includes all populations from the "Northern, 2n=38–40" plus "Northern mod 1, 2n=38–40" races, separated from the "Multiple Fissions, 2n=42–44" race. As riverine barriers also separate these chromosomal races, we do not attribute the observed differentiation to isolation-by-distance or the chromosome characterization for each race. Possible routes of migration and colonization are proposed.

Key words: allozyme variability, chromosomal races, central Chile, Liolaemidae, Squamata

INTRODUCTION

Liolaemus monticola Müller & Helmich, 1932, long considered as one of several simultaneously described subspecies of the *L. monticola* group, was recently elevated to species status (Torres-Pérez, 2004). It is a montane lizard endemic to Chile, distributed along the Mediterranean mountain ranges, between latitudes 30º and 36º S and at altitudes between 400 and 2300m (Donoso-Barros, 1966; Peters & Donoso-Barros, 1970). It displays a latitudinal gradient of increasing chromosomal polytypy from south to north, with extensive geographic variation in diploid chromosome number and shape, primarily in the number of centric fissions in the macro-chromosomes (pairs 1 to 4) in addition to other rearrangements (Lamborot, 1998). The most parsimonious explanation for the pattern observed in *L. monticola* is the origin of these races via fixation of successive chromosomal rearrangements that produce a linear series of races beginning with the presumed ancestral, 2n=32, with 12 macro-chromosomes and 20 micro-chromosomes, considered ancestral in *Liolaemus* (Lamborot, 1991, 1993, 2001; Lamborot & Alvarez-Sarret, 1989; Lamborot et al., 1979, 1981) and other iguanids (Gorman, 1973; Paull et al., 1976).

Elsewhere (Lamborot, 1991, 1993, 2001; Lamborot & Alvarez-Sarret, 1993), we have hypothesized that: 1) the "Southern, 2n=34" race was derived by a fixed reciprocal translocation that distinguishes this race from the "Primitive, 2n=32" race; 2) the "Southern, 2n=34" was ancestral to the "Northern, 2n=38–40" race, which retained the fixed translocation of the former, but is also clearly derived as evidenced by fixation of a pair 4 centric fission, the addition of another pair of micro-chromosomes, and polymorphic fission in pair 3; 3) the "Northern modified 1, 2n=38–40" race, as its name implies, originated from the "Northern, 2n=38–40" race, with two novel chromosomal mutations – a polymorphism for an enlarged chromosome 6 and a polymorphic pericentric inversion in chromosome 7 (Lamborot et al., 2003); 4) the "Northern modified 1, 2n=38–40" race gave rise to the "Multiple Fission (MF), 2n=42–44" race, that retains all the characteristic rearrangements of the former but is also polymorphic for fissions in pairs 1 and 2 (Lamborot et al., 2003). This race is considered the most derived and the most polymorphic of the *L. monticola* complex, and we have hypothesized that it represents a late colonization and differentiation event in more xeric habitats (Lamborot, 1998, 2001).

Several potential biogeographical barriers, such as the rivers Maipo and Yeso, separate the "Southern, 2n=34" and the "Northern, 2n=38–40" races (Lamborot, 1991, 2001; Lamborot & Alvarez-Sarret, 1993), and the river Aconcagua separates the "Northern, 2n=38–40" from the "MF, 2n=42–44" race (Lamborot, 1998; Lamborot et al., 2003). Narrow zones of hybridization have also been described between some races (Lamborot, 1991, 1993, 2001) not included in this study.

Based on population cytogenetic patterns, meiotic behaviour of chromosomal heterozygotes, the location and extent of hybrid zones (Lamborot, 1993, 2001), morphological analyses and the potential importance of the aforementioned riverine barriers (Lamborot & Eaton, 1992, 1997; Lamborot et al., 2003), we hypothesize that the "Northern, 2n=38–40" race was derived from the "South-

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ern, 2n=34" race, probably in the Coastal range that escaped the Pleistocene glaciations. These glaciations were extensive and in the penultimate episode glaciers were particularly well-developed in the Maipo and Aconcagua valleys (Brüggen, 1950; Caviedes, 1972; Formas, 1979; Huesser, 1966; Vuilleumier, 1971). The striking development of the glacial tongues could have acted as barriers, interrupting gene flow between the northern and southern populations, prior to the origin of the rivers in their present states (Lamborot, 1991, 2001). This may account for the pattern of chromosomal and morphological differentiation (Lamborot & Eaton, 1992, 1997; Lamborot, 2001; Lamborot et al., 2003). Initially, a genetically variable population of a "Southern, 2n=34-like" ancestor was widely distributed across the approximate present distribution of *L. monticola*, and subsequently became fragmented within glaciated river valleys during the Pleistocene. Several possible routes of migration and/or colonization have been proposed (Lamborot & Eaton, 1997; Lamborot, 2001; Lamborot et al., 2003).

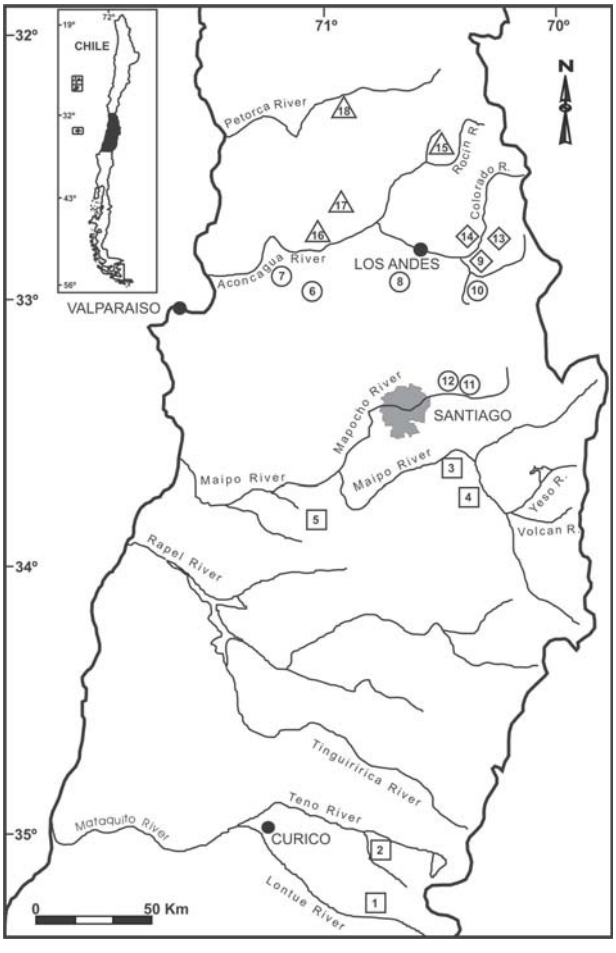

Fig. 1. Sampling localities in central Chile for four chromosomal races of *Liolaemus monticola*. Numbers correspond to localities sampled for the chromosomal and electrophoretic analysis. Squares: "Southern, 2n=34"; circles: "Northern, 2n=38–40"; diamonds: "Northern modified 1, 2n=38–40"; triangles: "Multiple Fissions, 2n=42–44". 1)Río Lontué, 2) Los Queñes, 3) Río Clarillo, 4) Maipo Sur, 5) Cerro Cantillana, 6) Cuesta La Dormida, 7) Cerro La Campana, 8) Cuesta Chacabuco, 9) Río Blanco, 10) Saladillo, 11) Farellones, 12) Yerba Loca, 13) Río Colorado South, 14) Río Colorado North, 15) Río Rocín, 16) Cabrería, 17) Mina Cerrillos, 18) Mina Hierro Viejo.

In this study, allozyme electrophoresis was used to estimate the pattern of genetic variation within and between four chromosomal races of *L. monticola*. The migration rate between races (Nm) was estimated to evaluate the potential dispersion from one population, or grouped populations, to another. Migration rates were then compared with previous morphological and molecular results (Lamborot & Eaton, 1992, 1997; Lamborot et al., 2003; Torres-Pérez et al., 2007). Allozymes may be a good tool for assessing genetic variability patterns when compared to other (e.g. morphological, mitochondrial) data sets (Avise, 2004). We tested for sequential founder events implied by patterns of chromosomal rearrangements among races, and assessed the potential role of barriers during glaciations in shaping present-day genetic diversity.

MATERIALS AND METHODS

Sampling

Samples of *Liolaemus monticola* used in this study were obtained from 18 localities (see Appendix 1 and Fig. 1). We grouped localities 3 (Río Clarillo) plus 4 (Maipo Sur), and 11 (Farellones) plus 12 (Yerba Loca), in order to have more lizards per sample. We took into account the lizards' geographical proximity, chromosome characteristics and lack of barriers (rivers) between them. All analyses were performed with 16 populations. All lizards were sacrificed by urethane injection in the pineal eye and were chromosomally characterized and assigned to one of the four races. Tissues (lungs, liver, skeletal muscle, stomach and duodenum) were preserved at −85 ºC for electrophoresis procedures. Voucher specimens are deposited in the collection of the Laboratorio de Citogenética, Departamento de Ciencias Ecológicas, Universidad de Chile (CUCH). Individual catalogue numbers designate specimens and their chromosomes.

Chromosomal data

Standard karyotypes for a subset of specimens collected in each locality were obtained from bone marrow, liver, spleen and testes. Karyotypes were dried using the colchicine-hypotonic pretreated air-drying method (Lamborot, 1993), and stained with Giemsa.

Electrophoresis methods

Samples of tissues used for horizontal starch-gel electrophoresis were homogenized following standard protocols (Murphy et al., 1996). Buffer, gel and running conditions at 4 ºC varied as follows: Tris Citrate pH 8.0 (90 V – 75 mA), 7–8 hours; Tris Citrate EDTA pH 8.0 (100 V – 75 mA), 7–8 hours; Tris Borate EDTA pH 8.0 (100 V – 35 mA), 8–10 hours. Gene products for the following 20 presumptive enzyme loci were analysed: aspartate aminotransferase (Ec 2.6.1.1, Aat-1), phosphoglucomutase (Ec 5.4.2.2, Pgm), glucose-6-phosphate isomerase (Ec 5.3.1.9, Gpi), α-esterase (Ec 3.1._._, α-Est), β-esterase (Ec 3.1._._, β-Est),

peptidase (Ec 3.4._._, Pep-a), α-mannosidase (Ec 3.2.1.24, α-Man), glutathione reductase (Ec 1.6.4.2, Gr), superoxide dismutase (Ec 1.15.1.1, Sod-1), lactate dehydrogenase (Ec 1.1.1.27, Ldh-a and Ldh-b), creatine kinase (Ec 2.7.3.2, Ck-1 and Ck-2), adenylate kinase (Ec 2.7.4.3, Ak-2), malate dehydrogenase (Ec 1.1.1.37, Mdh-1 and Mdh-2), glycerol-3-phosphate dehydrogenase (Ec 1.1.1.8, G3pdh), glucose dehydrogenase (Ec 1.1.1.118, Gcdh), aconitate hydratase (Ec 4.2.1.3, Acoh) and isocitrate dehydrogenase (Ec 1.1.1.42, Idh). Electromorphs of any given locus were considered homologous if they had the same mobility and were labelled in order of decreasing anodal or cathodal mobility.

Statistical analyses

Genotypic and allelic frequencies were determined by direct counts from allozyme phenotypes. Genetic variability included calculation of allelic frequencies, proportion of polymorphic loci (P), average of observed (H_o) and expected (H_e) heterozygosities and number of alleles per locus (A) (Appendix 2), using Genetix version 4.03 (Belkhir et al., 2004). Departures from Hardy-Weinberg expectations for all loci and all populations were assessed with Fisher exact tests using GenePop 3.1 (Raymond & Rousset, 1995). The distribution of allozyme diversity occurring within and between the *L. monticola* chromosomal races as groups, and populations as subgroups, was quantified as *F*-statistics using the analysis of molecular variance model (AMOVA; Excoffier et al., 1992) in Arlequin 3.0 (Excoffier et al., 2005). Population differentiation was assessed using the θ-estimator according to Weir & Cockerham (1984). We first calculated θst for all pairs of populations, and then hierarchically at the level of the four chromosomal races using Genetix 4.03. For detecting dissimilarity patterns, a non-metric multidimensional scaling analysis (MDS) for both populations and chromosomal races was performed in SYSTAT v. 8.0 based on a θst matrix according to Weir & Cockerham (1984). Using the θst parameter we calculated an indirect genetic estimate for number of migrants (Nm) among populations according to Wright (1931) as:

$$\theta st \approx 1/(4Nm+1).$$

Rogers' (1972) modified genetic distance was used to obtain a UPGMA phenogram using BIOSYS-1 (Swofford & Selander, 1989).

RESULTS

Pattern of allozyme variation

Five (25%) of the 20 presumptive loci analysed were monomorphic and fixed for the same electromorphs in all the samples studied (Ck-1, Mdh-1, Mdh-2, G3pdh, and Ldh-a). The allele frequencies at the other 15 variable loci and private alleles (marked in bold) are given in Appendix 2. At the population level, "private alleles" are those found in only one population (Barton & Slatkin, 1986); at the race level, those found in only one chromosomal race.

At a regional level, some features of the genetic diversity and diagnostic loci (at the 0.99% level) in the four races are reflected in allelic distributions that appear to define some geographically cohesive groups. First, Aat-1 appears polymorphic in all samples from the "Southern race" (samples 1–5, Fig. 2a) and fixed for electromorph "A" in the derived races, except that the two "Northern mod 1" race samples (13 and 14) share a private allele ("C") for this locus (Fig. 2a). Second, Pep-a is fixed for electromorph "B" in all samples of the "MF" race and variable in the remaining races, while the opposite situation is encountered for Gr (it is polymorphic in the "MF" race and in population 8 of the "Northern race", and invariable in the remaining races). The β-Est locus is monomorphic in the "Southern" race's Andean range (samples 1–4) but segregates two electromorphs in the Coast (sample 5) and is generally more polymorphic in the derived races (Fig. 2b). A similar pattern is evident in the Gcdh locus.

The overall mean number of alleles per locus (A) in the *L. monticola* populations ranged from 1.25 (Río Lontué) to 1.95 (Río Colorado Norte) (Appendix 2). The overall mean expected heterozygosity (H_e) ranged from 0.074 (Río Lontué) to 0.178 (Saladillo). The mean observed heterozygosity (H_o) ranged from 0.064 to 0.0185 in Río Lontué and Cerro La Campana, respectively. The highest mean percent polymorphism (P) was found in Cerro Cantillana and in Río Colorado Norte (60%), and the lowest was found in Río Lontué (25%). The values of polymorphic loci (P) and heterozygosity (H) within each race (data not shown) were similar in all races – "Northern, 2n=38–40": P=65%; "MF, 42–44": P=55%; "Northern mod 1": P=55%; "Southern, 2n=34": P=60%. The smallest heterozygosity values were found in the "Southern, 2n=34" populations (H_e=0.105; H_o=0.098), with similar values in the races located north of the Maipo River: "Northern, 2n=38–40" (H_e=0.170; H_o=0.167), "MF, 42–44" (H_e=0.129; H_o=0.141) and "Northern mod 1" (H_e=0.145; H_o=0.132).

Most samples were in Hardy-Weinberg equilibrium (HW in Appendix 2), except the following races and loci (* $P < 0.05$; ** $P < 0.01$): "Southern 2n=34" – sample 1, Aat (0.0371*); "Northern, 2n=38-40" – sample 6, β-Est (0.0207*) and Idh (0.0302*), sample 7, Sod (0.0152*), sample 10, Idh (0.0030**); "Northern, mod 1" – sample 13, β-Est (0.0013**), Pep (0.0175*) and α-Man (0.0224*), sample 14, α-Man (0.0250*) and Ldh-b (0.0258*).

Population structure and gene flow

Hierarchical variance analyses revealed that all differentiation tests at the different levels were significant (Table 1). The majority of the variance was found within populations (92.2%, F_{ST}=0.078, P<0.0001), with similar variance values among races (3.66%, F_{CT}=0.037, P<0.0001), and among populations within races (4.14%, F_{SC}=0.043, P<0.0001). According to the pairwise comparisons of the θst parameter, the non-hierarchical analysis (MDS) showed a dispersal of three groups of populations belonging to "Southern", "Northern" plus "Northern mod-1", and "Multiple Fissions" races into the multidimensional space (Fig. 3; Stress = 0.10181). This result was strongly supported (Stress < 0.0001) when the MDS analysis was performed grouping the populations into the four chromosomal races where the three above-mentioned groups appeared to be separated into the multidimensional space (Fig. 3).

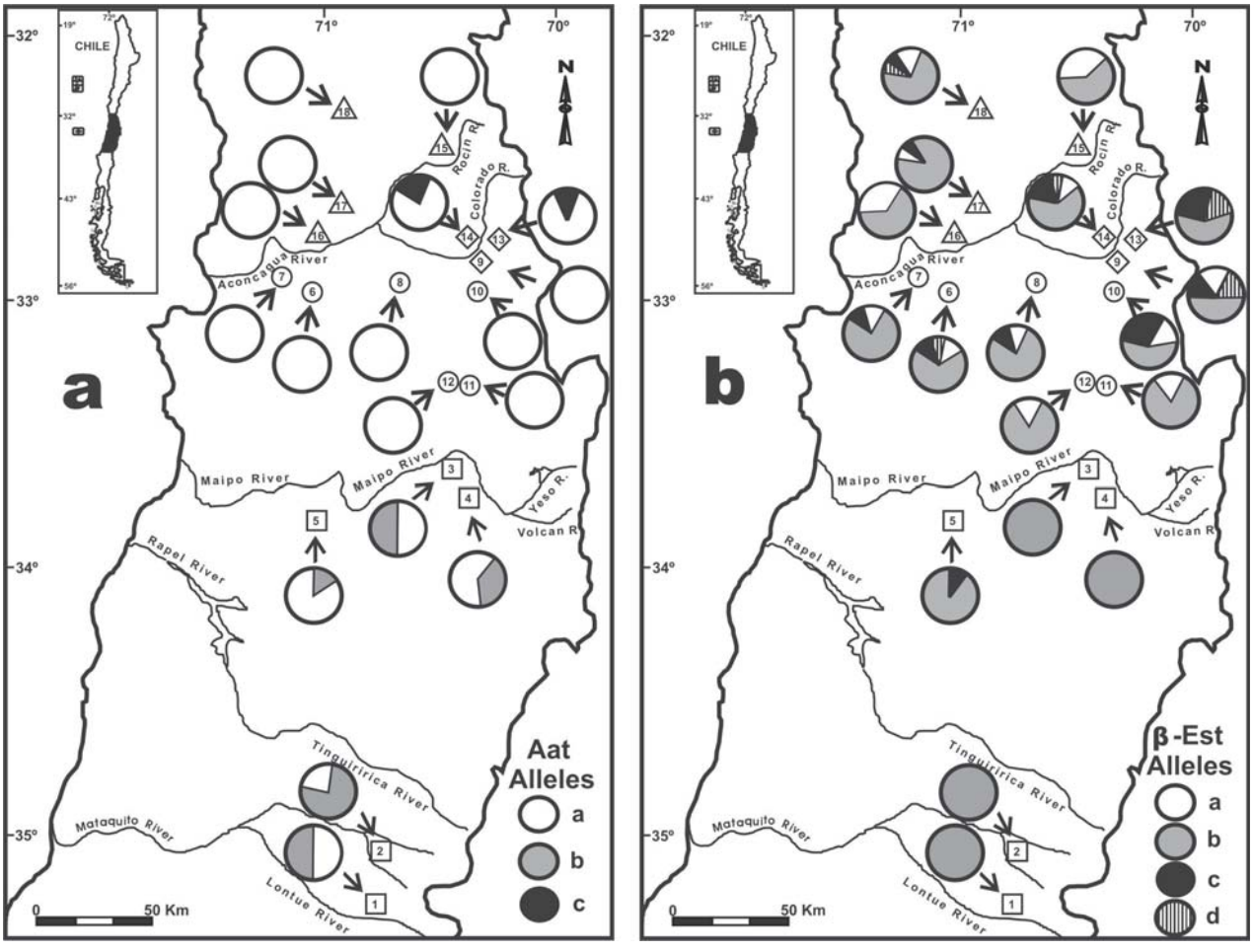

Fig. 2. Distribution of electromorph frequencies for two isoenzyme loci, a) s-Aat and b) β-Est, in four chromosome races of the *Liolaemus monticola* population samples.

The estimate of gene flow (Nm) based on Wright's method among all populations is 1.78 (data not shown). When the Nm values were obtained between pairs of races of geographic proximity (Table 2a) the lowest value was found for the "Southern, 2n=34" vs "Northern, 2n=38–40" (Nm=1.5). The highest Nm values were found between the "Northern" and "Northern mod 1" races (Nm = 13.3), and between the "Northern mod 1" vs the "MF" races (Nm=9.5).

Table 2b presents the Nm values within each race according to the ecodeme to denote a particular mountain range: Coastal, Transversal or Andean.

Figure 4 shows the results of the Nm values among all population races grouped by geographical proximity and chromosome characteristics with no barriers among them. In order to obtain more lizards per sample, samples with less than six lizards were omitted (sample 9, Río Blanco). The lowest Nm was found between the "Southern 2n=34" and the "Northern, 2n=38–40" races across the Maipo river (Nm=1.51): at the Andean range (sample 3+4 vs 11+12) the Nm is 1.3, while at the Coastal range (sample 5 vs 6+7) is 5.3. The Nm values across the Aconcagua river between the "Northern, 2n=38–40"and the "MF" races at the Coastal range (sample 6+7 vs 16+17) is 8.3; while the Nm values at the Andean range are higher between the "Northern", the "Northern mod 1" and the "MF" race populations. The highest Nm values are found between populations north of the Aconcagua and Colorado rivers:

Table 1. Hierarchical analysis of genetic variance and Fst (AMOVA) for four *Liolaemus monticola* chromosomal races. The amount of variance was assessed between groups (i.e. pairs of chromosomal races), between populations within chromosomal races, and within populations.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation indexes	*P*-value
Between races	3	62.013	0.09439	3.66%	F_{CT} = 0.037	< 0.0001
Between populations within races	12	75.271	0.10693	4.14%	F_{SC} = 0.043	< 0.0001
Within populations	590	1404.082	2.37980	92.20%	F_{ST} = 0.078	< 0.0001
Total	605	1541.366	2.58112			

Table 2. Nm estimates across several hierarchical levels of divergence: a) across four *Liolaemus monticola* chromosomal races; b) the ecodemes within each race denote a particular mountain range: Coastal, Transversal or Andean.

a)

	Northern	Northern mod 1	MF
Southern	1.51	1.64	1.59
Northern	-	13.27	7.69
Northern Mod	-	-	9.52

b)

	Southern Coast	Northern Coast	Northern Transversal	Northern Andes	Northern mod 1	MF Andes	MF Coast
Southern Andes	2.38	1.09	0.84	0.93	1.18	0.95	1.08
Southern Coast	-	5.31	2.91	3.30	4.06	3.96	5.30
Northern Coast	-	-	21.35	35.70	11.87	7.95	7.55
Northern Transversal	-	-	-	22.79	7.70	6.63	5.06
Northern Andes	-	-	-	-	11.79	7.91	5.95
Northern mod 1	-	-	-	-	-	13.83	8.64
MF Andes	-	-	-	-	-	-	234.88

Cerrillos plus Cabrería vs Río Rocín (Nm >> 100) belonging to the "MF" race, Río Rocín (MF race) vs Río Colorado Norte ("Northern mod 1" race) (Nm = 30.6).

Genetic distances and phenetic groups

For all populations of *L. monticola*, modest values of Rogers' distances (D) were estimated among samples (Fig. 5), ranging from 0.046 (sample 15 *vs* 17, from the "MF" race) to 0.238 (sample 2 vs 10, from the "Southern" race Andean range vs the "Northern" race). The UPGMA phenogram revealed two main clusters (Fig. 5a): one cluster corresponds to all "Southern, 2n=34" race populations (1–5 in Fig. 1), and the second cluster includes all remaining races (Fig. 5a). Within this second group, two subclusters are recognized: one includes all four "MF" (samples 15–18) and the other the "Northern" and the "Northern mod 1" races (samples 6–14). When we repeated the UPGMA by collapsing the Rogers' D matrix into geographic subregions within each race, the phenogram (Fig. 5b) showed a congruence of chromosome groups with the hypothesized Maipo river and Aconcagua river barriers, and is generally concordant with Fig. 5a.

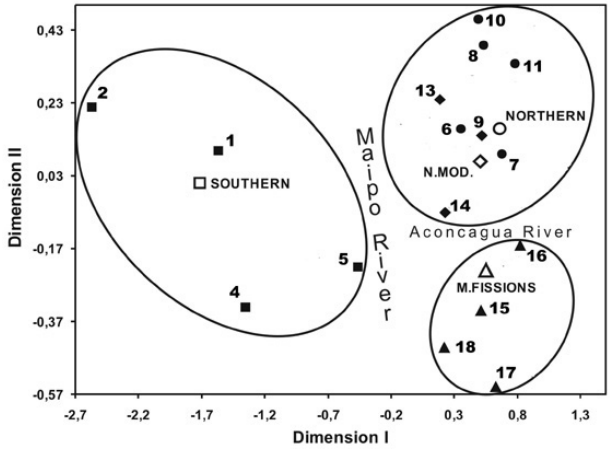

Fig. 3. Bidimensional ordination for both populations and chromosomal races of the *Liolaemus monticola* complex using non-metric multidimensional scaling (MDS). Symbols are similar to those in Figure 1. Full symbols represent the analysis performed using the 16 populations studied and empty symbols represent the analysis by chromosomal races (by grouping the populations into their respective chromosomal races).

DISCUSSION

Chromosomal evolution

Liolaemus monticola provides an interesting link between chromosomal polytypy and possible speciation. The linearly arranged karyotypic variation is corroborated by the increased complexity from south to north. This clinal pattern resembles Hall's "cascade model of speciation" (Hall, 1973, 1980, 1983), the "chain process" (White, 1978) or "primary chromosomal allopatry" (King, 1981) hypotheses. However, there are a variety of chromosome speciation models, and many are based on restrictive assumptions (see Sites & Reed, 1994; Sites & Moritz, 1987; King, 1981, 1993).

One interesting aspect in the *L. monticola complex* is the effect of chromosomal rearrangements on the fitness of heterozygous hybrids even when chromosomal rearrangements have little or no effect on hybrid fitness (Lamborot, 1993). The comparison made on spermatocyte bivalents shows that chiasmata patterns in the "Southern, 2n=34" race (with nonfissioned macro-chromosomes) are invariably intersticial and distal, while chiasmata for

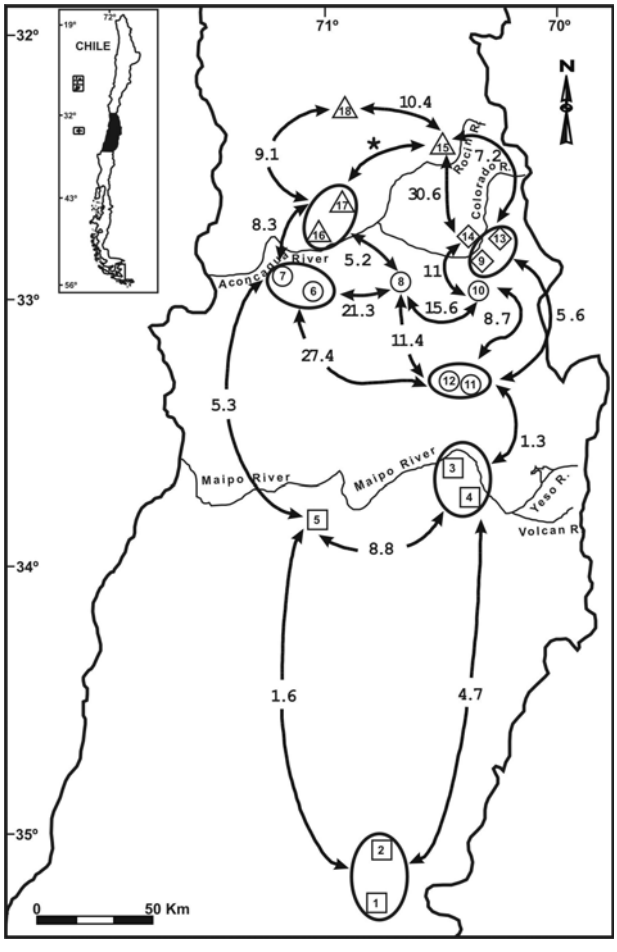

Fig. 4. Distribution of the estimated number of migrants per generation (Nm) for grouped populations by geographic proximity. For the geographic origin see Figure 1 and Material and Methods. *Negative values for èst due to surrounding errors in calculations can be interpreted as equal to zero (Long, 1986), then Nm >> 100. Negative values were found between two "MF" populations (sample 15 Río Rocín, and sample 16 + 17 Mina Cerrillos + Cabrería).

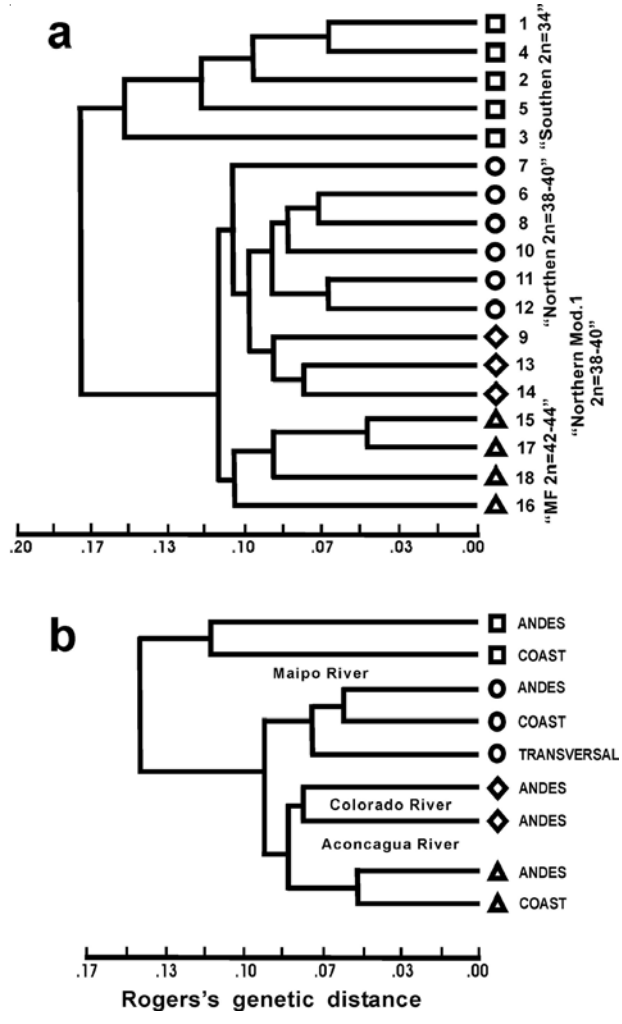

Fig. 5. UPGMA phenograms based on a) Rogers' distance matrix among all pairwise *Liolaemus monticola* samples, and b) all pairwise *L. monticola* chromosome races grouped by geographic subregions. For geographic origin see Fig. 1, and Materials and Methods.

the "Northern, 2n=38–40" race were always terminal. The hybrid "Northern x Southern" chiasmata are intermediate (Lamborot, 1991).

In males, this disproportionately lower chiasma frequency per unit length constitutes a means of reduction of recombination for the "Northern" and the "MF" races. This fact prevents the break-up of adaptative linkage groups, which allowed a chromosomally derived founder population to radiate into a different (more xeric) habitat without loss of reproductive potential throughout excessive segregations that might reduce gene flow through the suppression of recombination. From this point of view, it seems that Robersonian rearrangements, such as fissions, might retain adaptative gene combinations by limiting the amount of variability resulting from intrachromosomal recombination. On the other hand, these would enhance variability by interchromosomal recombination, preserving gene sequences relatively well adapted to a new environment (Lamborot, 1991). In conclusion, considerable additional data will be required before the role of chromosomal rearrangements in speciation can be confidently evaluated (Rieseberg, 2001).

The *L. monticola* complex has been compared to the *Sceloporus grammicus* complex in Mexico because the two are in many respects independent "experimental" replicates, exhibiting strikingly similar phenomena at equivalent hierarchical levels (i.e. the maintenance of within-race chromosomal polymorphisms, parapatric hybrid zones between races, etc.; Sites & Reed, 1994). One advantage of studying the *L. monticola* complex is its north–south linear distribution of chromosome races, which is much less complex than the distribution of the *S. grammicus* chromosome races. In fact, a recent study using both chromosomes and allozymes in *S. grammicus* showed significant differences allowing delimitating species that were previously recognized as chromosomal races (Marshall et al., 2006). These similarities and differences notwithstanding, additional detailed phylogenetic and phylogeographic studies of both complexes, based

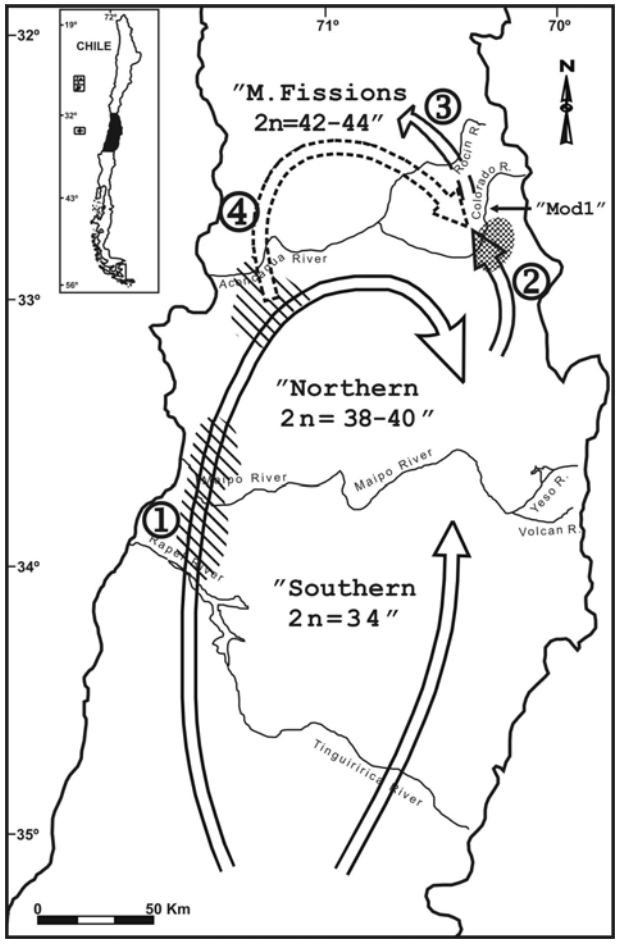

Fig. 6. Hypothesized glacial and postglacial migration and colonization routes from refugia (as hatched areas) in the Coastal Range. First, origin of the "Northern, 2n=38–40" race (north of the Maipo river) by a Coastal range expansion (route 1) and then expansion upward in elevation following glacial retreat. Second, the origin and spread of the "Northern mod 1" race (route 2) in the Andean range and into the "MF" race's actual distribution (route 3). Route 4 (dotted line): possible route of origin of the "MF" race by colonization across the Aconcagua river in the Andean range from the Coastal range refuge, and then eastward expansion. Dotted area denotes possible hybridization zone.

on independent markers, will be needed to unambiguously test the direction and tempo of chromosome evolution.

Pattern of genetic variability

The allozyme genetic polymorphism in *L. monticola* in central Chile is relatively high and the level of heterozygosity in each chromosomal race (H_o=0.099–0.168) is larger than that of Nevo (1978), Gorman et al. (1978), Hall & Selander (1973) and Sites et al. (1988), but less than the average calculated by Mendoza-Quijano et al. (1998) for reptiles. The highest values of heterozygosity in *L. monticola* are found in the "Northern, 2n=38–40" race (H_e=0.170; H_o=0.167), whereas the lowest ones are observed in the "Southern, 2n=34" race (H_e=0.105; H_o = 0.099). When the heterozygosity is compared between geographical subgroups, within the "Southern, 2n=34" race, the Andean range samples (H_e=0.086; H_o=0.085) are considerably lower than those from the Coastal range (H_e=0.134; H_o=0.136). The Coastal range has been considered a refugium for some species during periods of glacial advance (Formas, 1979).

The H_o value of the "Northern mod 1" race, 0.132, represents the lowest value among the derived races. It is possible that the "Northern mod 1, 2n=38–40" race currently represents a hybrid zone area between the "Northern, 2n=38–40" and the "MF, 2n=42–44" races (Fig. 6, route 3). The geographic location of this race is intermediate between these two, and a "hybridization hypothesis" would explain 1) the geographic location, 2) the large number of loci that deviate from the Hardy-Weinberg genotype ratios (five out of 10 of the total samples are not in equilibrium), 3) the presence of polymorphisms for two chromosome rearrangements in the "MF, 2n=42–44" race and 4) the large number of private alleles and the presence of three out of six private alleles in the 15 loci analysed from the total sample (Aat, Pep-A and Ck-2). Such alleles of mutational origin are called "hybridzymes" (Woodruff, 1989). Private allelic richness is a convenient feature of how distinct a population is from other populations.

Population structure and barriers to gene flow

The θst estimates and genetic distances show that, at the scale of this study, the genetic variation in *L. monticola* is distributed into geographically coherent chromosomal races and the riverine barriers that separate them (Table 1, Figs 1, 3 and 5).

Based on genetic distances, one distinct group includes all "Southern, 2n=34" race populations south of the Maipo river (Fig. 5a,b) and a second includes all races north of the Maipo river. These races are all distinct from each other. Based on MDS, only three well-resolved groups are distinguished: the "Southern", the "MF" and a third grouping the "Northern, 2n=38–40" race with "Northern mod 1", but this would not be unexpected given the geographic proximity of these localities, and the possible hybrid zone status of these races.

When samples are grouped using both geographical proximity and chromosome characteristics as guides, the analysis of Nm (Fig. 4) shows that the lowest value of gene flow is between the "Southern, 2n=34" and "Northern, 2n=38–40" races, from the Andean range (Nm=1.3). The Maipo river is considered an effective barrier to dispersal for the conservative karyotype that characterizes the "Southern" race, except for a local hybrid zone not included in this study. Within the Andean range, this river appears to be a more effective barrier (Nm=1.3) compared to the Coastal range (Nm=5.3). In contrast, the Aconcagua river provides a stronger barrier at the mouth (Nm= 8.3) in the Coastal range, than near the headwaters near the Andean range (Nm=11.0). Thus it would appear that both the Maipo river and the Aconcagua river have played important roles in the differentiation of populations and chromosomal races of *L. monticola*. The

Maipo river is a stronger barrier near the Andes compared to its mouth and the Coast. This is the opposite of what would be expected by the "riverine barrier hypothesis" (Patton et al., 1994). However the upper reaches of some other rivers such as the Colorado (samples 9, 13 and 14) and the Rocín (samples 14 and 15), for example (Fig. 4), do not separate chromosome races, suggesting limited post-glacial expansion of lizards across smaller headwater streams. This same pattern has been shown for some mammal groups in the Amazonian tributary basins (Patton et al., 1994).

In spite of the low genetic distance among chromosome races, there is a concordance with race boundaries and large differences in allele frequencies. Nevertheless, private alleles indicated limited introgression and suggest range fragmentation produced by late Pleistocene geological events.

In addition, phylogeographic analyses using the mitochondrial cytochrome *b* gene in the chromosomal races involved in this study (Torres-Pérez et al., 2007), supported the existence of two well-resolved groupings in *Liolaemus monticola*: the first most basal major clade included all the "Southern, 2n=34" samples south of the Maipo river. The second clade included samples of the remaining chromosomally derived races, with no significant differences between the "Northern" and the "Multiple Fissions" races. This suggests that the nuclear co-dominant markers (e.g. allozymes) detect more variability within and among populations allowing the discrimination of patterns of gene flow at a finer scale. Non-congruent molecular patterns between nuclear and mitochondrial markers have been reported (Avise, 2004), mainly because mitochondria are maternally inherited, introducing a bias in describing only a portion of the complete processes occurring in the *L. monticola* species.

Possible route of migration and/or colonization

Based on distributions in five nuclear markers (Pep-A, GR, Gcdh, Aat and α-Est, Fig. 2a,b), we propose a model of colonization for the *L. monticola* complex in this region of Chile. We hypothesize an initial colonization from south to north which established a "Southern, 2n=34" race occupying almost the same geographical area as that focused on in this study, and subsequent colonizations and origins of the derived chromosomal races. Figure 6 presents a hypothesis for the sequence of events and is consistent with chromosomal rearrangements (Lamborot, 1991, 1993, 1998, 2001; Lamborot & Alvarez-Sarret, 1993), morphological (Lamborot & Eaton, 1992, 1997; Lamborot et al., 2003), and genetic differentiation in the *L. monticola* populations (this study; Torres-Pérez et al., 2007).

These analyses indicated a high degree of consistency between chromosomal, morphological and genetic differentiation in populations of the *L. monticola* chromosome races. However, the genetic distances obtained in this study are lower than those obtained for chromosomes and morphology. This suggests that allozymes are nearly neutral genetic markers and are best for estimating gene flow, reflecting the effect of history and chance, whereas patterns of variation in many morphological characters may reflect the effects of natural selection. In summary we emphasize concordance of markers and race boundaries, and only limited introgression.

This paper is part of a larger project exploring variation at different levels: chromosomal, morphological and molecular. Ultimately, we expect to be able to correlate these different data sets for a better understanding of population divergence, coupled with chromosomal divergence, introgression, hybrid zones and the possible evolutive associations.

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APPENDIX 1

Localities, sample sizes, altitude, chromosome races, and mountain ranges for the lizards of the *Liolaemus monticola* complex used in this study

Chromosomal race 2n=34

RIO LONTUE (35°08'S, 71°18'W, altitude 1200 m, mountain range Andes): CUCH-2108, 2109, 2382– 2388; LOS QUEÑES (35°07'S, 70°52'W, altitude 1200 m, mountain range Andes): CUCH-1459, 1460, 1464, 1468, 2312, 2583, 2585, 2635–2646, 2586, 2584; RIO CLARILLO (33°43'S, 70°30'W, altitude 1300 m, mountain range Andes) :CUCH-2654, 2655; MAIPO SUR (33°39'S, 70°22'W, altitude 1300 m, mountain range Andes): CUCH-2661–2667, 2765, 2766–2774; CERRO CANTILLANA (33°57'S, 70°56'W, altitude 1300 m, mountain range Coast): CUCH-1310–1316, 1318, 1322, 1326, 1327, 1323, 1331–1333, 1335, 1336, 1341.

Chromosomal race 2n=38–40

CUESTA LA DORMIDA (33°03'S, 71°02'W, altitude 1300 m, mountain range Coast): CUCH-1408, 1410, 1450–1453, 1455, 2149, 2156, 2492, 2496, 2495, 2499, 2487, 2494, 2524, 2525, 2527–2535; CERRO LA CAMPANA (32°57'S, 71°07'W, altitude 1400 m, mountain range Coast): CUCH-1381– 1392; CUESTA CHACABUCOe 32°56'S, 70°44'W, altitude 1100 m, mountain range Transversal): CUCH-2078–2086, 2279, 2089, 2209–2216, 2278, 2282, 2219–2222; RIO BLANCO (32°55'S, 70°16'W, altitude 1450 m, mountain range Andes): CUCH-2137–2139, 2443, 2446; SALADILLO (32°58'S, 70°18'W, altitude 1450 m, mountain range Andes): CUCH-2285–2290, 2447–2461, 2463, 2464, 2467, 2468, 2475; FARELLORES (32°20'S, 70°20'W, altitude 1400 m, mountain range Andes): CUCH-1422, 1670–1672, 1675–1679, 1681 –1686, 1691; YERBA LOCA (33°21'S, 70°20'W, altitude 1800 m, mountain range Andes): CUCH-1425, 1428, 1435, 1438, 1660–1663, 1668, 1669; RIO COLORADO SOUTH (32°53'S, 70°20'W, altitude 1550 m, mountain range Andes): CUCH-2540–2542, 2559–2563, 2565, 2567, 2568, 2570–2574, 2578–2582, 2591, 2592, 2599–2601; RIO COLORADO NORTH (32°52'S, 70°22'W, altitude 1550 m, mountain range Andes): CUCH-2539, 2543–2546, 2551–2558, 2613, 2615–2622.

Chromosomal race 2n=42–44

RIO ROCIN (32°32'S, 70°25'W, altitude 1600 m, mountain range Andes): CUCH-2090–2097, 2099, 2100, 2170–2185; CABRERIA (32°56'S, 71°02'W, altitude 600 m, mountain range Coast): CUCH-2373–2379; MINA CERRILLOS (32°28'S, 71°08'W, altitude 1400 m, mountain range Coast): CUCH-2357–2368; MINA HIERRO VIEJO (32°18'S, 70°58'W, altitude 350 m, mountain range Coast): CUCH-1354–1362, 1807–1818.

APPENDIX 2. Electromorph frequencies and genetic variability parameters for 15 polymorphic loci in 16 locality samples of the *Liolaemus monticola* complex from Central Chile.

	Population															
Locus	1	2	3+4	5	6	7	8	9	10	11+12	13	14	15	16	17	18
AAT1 (N)	9	21	19	18	26	14	25	4	26	26	26	22	26	7	12	21
A	0.5000	0.2381	0.6316	0.8611	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9038	0.8409	1.0000	1.0000	1.0000	1.0000
B	0.5000	0.7619	0.3684	0.1389	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	*0.0962*	*0.1591*	0.0000	0.0000	0.0000	0.0000
HW	0.0371	0.5375	0.3171	0.2683	–	–	–	–	–	–	0.1894	1.0000	–	–	–	–
PGM1 (N)	5	21	19	18	26	13	25	5	26	26	26	22	26	7	12	21
A	0.0000	0.0000	0.0000	*0.0556*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
B	0.0000	0.0476	0.0000	0.1389	0.0000	0.0769	0.0000	0.0000	0.0192	0.0192	0.0000	0.0227	0.0000	0.0000	0.0000	0.0952
C	1.0000	0.9524	1.0000	0.8056	0.9231	0.9231	0.9400	1.0000	0.9808	0.9615	1.0000	0.9091	1.0000	1.0000	0.9583	0.9048
D	0.0000	0.0000	0.0000	0.0000	0.0769	0.0000	0.0600	0.0000	0.0000	0.0192	0.0000	0.0682	0.0000	0.0000	0.0417	0.0000
HW	–	1.0000	–	1.0000	1.0000	1.0000	1.0000	–	–	1.0000	–	1.0000	–	–	–	1.0000
PGI1 (N)	9	20	17	18	25	12	24	5	26	26	26	21	26	7	12	21
A	0.0000	0.0000	0.0882	0.1111	0.0600	0.0833	0.0000	0.0000	0.0000	0.0000	0.0000	0.0238	0.0192	0.0000	0.0000	0.0714
B	1.0000	1.0000	0.9118	0.8889	0.9400	0.9167	1.0000	1.0000	1.0000	1.0000	1.0000	0.9762	0.9808	1.0000	1.0000	0.9286
HW	–	–	1.0000	0.1699	1.0000	1.0000	–	–	–	–	–	–	–	–	–	1.0000
αEST (N)	9	21	19	17	24	14	24	5	25	26	24	22	26	7	11	18
A	0.0000	0.0000	0.0000	0.0000	0.0833	0.1071	0.1042	0.1000	0.1000	0.0962	0.0625	0.0909	0.0000	0.0000	0.0000	0.0000
B	0.0000	0.0238	0.1053	0.1176	0.3750	0.3929	0.4583	0.3000	0.4800	0.4423	0.3125	0.4091	0.4423	0.2857	0.5000	0.3056
C	1.0000	0.9762	0.8947	0.8824	0.5417	0.5000	0.4375	0.6000	0.4200	0.4615	0.6250	0.5000	0.5577	0.7143	0.5000	0.6944
HW	–	–	1.0000	1.0000	0.4002	0.0637	1.0000	1.0000	0.9071	0.6846	0.6174	0.3956	0.6904	1.0000	0.0931	0.1239
βEST (N)	7	20	19	18	24	14	24	5	25	23	25	22	25	5	11	20
A	0.0000	0.0000	0.0000	0.0000	0.1458	0.1429	0.0833	0.2000	0.0000	0.2174	0.0000	0.0909	0.0600	0.4000	0.0455	0.1500
B	1.0000	1.0000	1.0000	0.9444	0.6875	0.7500	0.8333	0.5000	0.5800	0.7826	0.5800	0.7045	0.8000	0.6000	0.9091	0.7500
C	0.0000	0.0000	0.0000	0.0556	0.1042	0.1071	0.0833	0.1000	0.2600	0.0000	0.2200	0.1818	0.1200	0.0000	0.0455	0.0500
D	0.0000	0.0000	0.0000	0.0000	0.0625	0.0000	0.0000	0.2000	0.1600	0.0000	0.2000	0.0227	0.0200	0.0000	0.0000	0.0500
HW	–	–	–	1.0000	0.0207	1.0000	1.0000	0.1077	0.8174	0.5408	0.0013	0.5627	1.0000	1.0000	1.0000	1.0000
PEP (N)	5	20	19	18	22	14	25	5	26	26	26	21	26	7	11	21
A	0.1000	0.0750	0.0526	0.0833	0.2500	0.1786	0.3200	0.1000	0.1731	0.1731	0.1154	0.0000	0.0000	0.0000	0.0000	0.0000
B	0.9000	0.9250	0.9474	0.9167	0.7045	0.8214	0.6400	0.9000	0.8269	0.8269	0.8846	0.9524	1.0000	1.0000	1.0000	1.0000
C	0.0000	0.0000	0.0000	0.0000	*0.0455*	0.0000	*0.0400*	0.0000	0.0000	0.0000	0.0000	*0.0476*	0.0000	0.0000	0.0000	0.0000
HW	–	1.0000	1.0000	1.0000	0.7881	1.0000	0.6923	–	1.0000	1.0000	0.0175	1.0000	–	–	–	–
αMAN (N)	8	17	17	13	24	13	24	5	23	22	23	21	19	6	11	15
A	0.1875	0.0000	0.0588	0.1923	0.2083	0.2308	0.0625	0.2000	0.1957	0.2955	0.0435	0.0476	0.0789	0.2500	0.1364	0.0333
B	0.8125	1.0000	0.9412	0.8077	0.7917	0.7692	0.9375	0.8000	0.8043	0.7045	0.9565	0.9524	0.9211	0.7500	0.8636	0.9667
HW	1.0000	–	1.0000	0.3729	0.5411	1.0000	1.0000	1.0000	0.1633	0.6124	0.0224	0.0250	1.0000	1.0000	1.0000	–
GR (N)	7	20	19	17	19	9	20	2	26	22	20	18	21	6	11	19
A	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	*0.0750*	0.0000	0.0000	0.0000	0.0000	0.0000	*0.1190*	*0.0833*	*0.1818*	***0.2368***
B	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9250	1.0000	1.0000	1.0000	1.0000	1.0000	0.8810	0.9167	0.8182	0.7632
HW	–	–	–	–	–	–	1.0000	–	–	–	–	–	1.0000	–	1.0000	0.5281

Appendix 2 (cont.)

	Population															
Locus	1	2	3+4	5	6	7	8	9	10	11+12	13	14	15	16	17	18
SOD4 (N)	9	14	17	14	19	8	22	4	19	23	19	16	19	7	11	16
A	1.0000	1.0000	1.0000	1.0000	1.0000	0.7500	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
B	0.0000	0.0000	0.0000	0.0000	0.0000	*0.2500*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
HW	–	–	–	–	–	0.0152	–	–	–	–	–	–	–	–	–	–
LDHa (N)	9	18	19	18	24	13	22	5	25	26	25	20	25	7	12	21
A	0.0000	0.0556	0.0000	0.1111	0.0833	0.0769	0.0682	0.0000	0.1000	0.1154	0.0400	0.0500	0.1200	0.0714	0.1250	0.0238
B	1.0000	0.9444	1.0000	0.8889	0.9167	0.9231	0.9318	1.0000	0.9000	0.8846	0.9600	0.9500	0.8800	0.9286	0.8750	0.9762
HW	–	1.0000	–	1.0000	1.0000	1.0000	1.0000	–	0.1968	1.0000	1.0000	0.0258	0.2865	–	1.0000	–
CK2 (N)	9	21	19	18	26	13	25	5	25	26	26	22	23	6	12	21
A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9808	1.0000	0.9783	1.0000	1.0000	1.0000
B	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	*0.0192*	0.0000	*0.0217*	0.0000	0.0000	0.0000
HW	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
AK2 (N)	5	19	19	18	26	13	24	5	25	26	26	21	20	7	12	21
A	0.9000	0.8947	0.9211	0.8333	0.7885	0.8462	0.7292	0.6000	0.7400	0.6154	0.7308	0.7857	0.8750	0.8571	0.9583	0.7381
B	0.1000	0.1053	0.0789	0.1667	0.2115	0.1538	0.2708	0.4000	0.2600	0.3846	0.2692	0.2143	0.1250	0.1429	0.0417	0.2619
HW	–	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6392	0.0958	1.0000	0.5300	0.2412	1.0000	–	1.0000
GCDH (N)	5	18	17	15	20	11	20	4	24	19	20	17	18	7	12	17
A	0.0000	0.0000	0.0000	0.1000	0.0750	0.0000	0.2250	0.0000	0.2083	0.1316	0.2250	0.0882	0.1944	0.3571	0.1667	0.0588
B	0.8000	0.8611	0.7941	0.8000	0.8000	0.6818	0.6500	0.8750	0.6042	0.7368	0.6750	0.8235	0.7778	0.6429	0.7500	0.7353
C	0.2000	0.1389	0.2059	0.1000	0.1250	0.3182	0.1250	0.1250	0.1875	0.1316	0.1000	0.0882	0.0278	0.0000	0.0833	0.2059
HW	1.0000	1.0000	1.0000	1.0000	1.0000	0.4818	0.3825	–	0.2534	0.7482	0.2245	0.1945	1.0000	0.1058	1.0000	0.7188
ACOH (N)	5	15	16	8	21	9	19	4	22	17	16	17	15	7	10	14
A	0.0000	0.0667	0.0313	0.0000	0.0952	0.0000	0.0789	0.1250	0.0909	0.0294	0.0938	0.1176	0.0667	0.0000	0.0000	0.0000
B	1.0000	0.9333	0.9375	0.9375	0.8810	0.9444	0.8684	0.8750	0.8864	0.9706	0.9063	0.8529	0.9000	1.0000	0.9500	0.9286
C	0.0000	0.0000	0.0313	0.0625	0.0238	0.0556	0.0526	0.0000	0.0227	0.0000	0.0000	0.0294	0.0333	0.0000	0.0500	0.0714
HW	–	1.0000	1.0000	–	1.0000	–	1.0000	–	1.0000	–	1.0000	1.0000	1.0000	–	–	1.0000
IDH1 (N)	5	14	14	9	21	8	14	3	21	15	15	16	15	7	10	12
A	0.0000	0.1429	0.0000	0.0000	0.0714	0.0000	0.0714	0.0000	0.1667	0.0333	0.0000	0.0938	0.1333	0.0714	0.0500	0.0000
B	1.0000	0.8571	0.8929	0.8889	0.8810	1.0000	0.8571	1.0000	0.7143	0.9333	1.0000	0.8438	0.8667	0.9286	0.9500	0.8750
C	0.0000	0.0000	0.1071	0.1111	0.0476	0.0000	0.0714	0.0000	0.1190	0.0333	0.0000	0.0625	0.0000	0.0000	0.0000	0.1250
HW	–	1.0000	0.1099	1.0000	0.0302	–	0.2183	–	0.0030	1.0000	–	0.2991	1.0000	–	–	0.1309
He	0.0742	0.0770	0.0904	0.1336	0.1665	0.1625	0.1630	0.1309	0.1783	0.1492	0.1353	0.1486	0.1236	0.1193	0.1087	0.1382
Ho	0.0643	0.0799	0.1004	0.1357	0.1697	0.1853	0.1620	0.1350	0.1614	0.1654	0.1118	0.1516	0.1338	0.1176	0.1377	0.1608
P	25	45	45	60	55	55	55	35	50	50	50	60	55	40	50	55
A	1.2500	1.4500	1.5000	1.7000	1.9000	1.6500	1.8500	1.5000	1.7500	1.7000	1.6500	1.9500	1.7500	1.4000	1.6000	1.7000

(N) = Sample number; HW = Hardy-Weinberg equilibrium; Expected (H_e) and observed (H_o) heterozygosity; P = Percentage of polymorphic loci at the 99%-- level; A = average number of alleles per locus. Private alleles are in italics.