Evolution, 57(4), 2003, pp. 913–926

GENETIC AND GEOGRAPHIC DIFFERENTIATION IN THE RIO NEGRO TUCO-TUCO (*CTENOMYS RIONEGRENSIS*): INFERRING THE ROLES OF MIGRATION AND DRIFT FROM MULTIPLE GENETIC MARKERS

GABRIELA WLASIUK,[1,2] JOHN CARLOS GARZA,[3,4,5] AND ENRIQUE P. LESSA[1,6]
[1]*Laboratorio de Evolución, Facultad de Ciencias, Montevideo 11400, Uruguay*
[2]*E-mail: wlasiuk@fcien.edu.uy*
[3]*Museum of Vertebrate Zoology, University of California–Berkeley, Berkeley, California 94720*
[4]*Southwest Fisheries Science Center, Santa Cruz Laboratory, Santa Cruz, California 95060*
[5]*E-mail: carlos.garza@noaa.gov*
[6]*E-mail: lessa@fcien.edu.uy*

Abstract.—Among tuco-tucos, *Ctenomys rionegrensis* is especially amenable to the study of the forces driving population differentiation because of the restricted geographic range it occupies in Uruguay. Within this limited area, the Rio Negro tuco-tuco is limited to sandy soils. It nonetheless exhibits remarkable variation in pelage color, including melanic, agouti, and dark-backed individuals. Two hypotheses have been put forth to explain this pattern: (1) local differentiation and fixation of alternative pelage types by genetic drift under limited gene flow; or (2) fixation by natural selection that may take place even in the presence of gene flow. A previous allozyme study rejected the genetic drift hypothesis on the basis of high inferred levels of migration. New estimates of gene flow from microsatellites and mitochondrial cytochrome *b* sequences were obtained for *C. rionegrensis* populations to further test these hypotheses. Much lower levels of gene flow were estimated with these more sensitive markers. Microsatellite-based estimates of gene flow are close to zero and may come closest to estimating current levels of migration. A lack of equilibrium between migration and genetic drift is also strongly suggested by the absence of an isolation-by-distance pattern found in all three genetic datasets. The microsatellite genotype data show that the species is strongly structured geographically, with subpopulations constituting distinct genetic entities. If current levels of gene flow are very low, as indicated by the new data, the local fixation of alternative alleles, including those responsible for pelage color polymorphism, is possible by drift alone. A scenario is thus proposed in which the species expanded in the recent past from a more restricted geographic range and has subsequently differentiated in near isolation, with genetic drift possibly playing a primary role in overall genetic differentiation. The local fixation of pelage color types could also be due to drift, but selection on this trait cannot be ruled out without direct analysis.

Key words.—Chromatic polymorphism, *Ctenomys rionegrensis*, gene flow, microsatellites, phylogeography, range expansion, tuco-tuco.

Received December 14, 2001. Accepted November 22, 2002.

A central discussion in studies of differentiation and speciation has been the relative importance of natural selection and genetic drift in promoting reproductive isolation (Coyne 1992). Recent studies of diverse taxa suggest that natural selection associated with shifts in ecology can lead to rapid divergence and affect the rate of evolution of reproductive isolation, even in the presence of considerable amounts of gene flow (reviewed in Orr and Smith 1998; Schluter 1998). Evidence is thus emerging that ecological processes can play a pivotal role in speciation (Bush 1994; Kondrashov et al. 1998; Orr and Smith 1998).

South American tuco-tucos (genus *Ctenomys*) are a diverse group of subterranean rodents with about 60 extant species (Reig et al. 1990; Lacey et al. 2000). Extensive diversification began only in the Pleistocene and, as such, *Ctenomys* is likely the most rapidly speciating genus of extant mammals (Reig 1989).

An assessment of population structure and processes, especially the estimation of critical population parameters, such as gene flow, could be of key importance in understanding the differentiation dynamics of the group. The role of gene flow in shaping population structure depends of its interplay with natural selection and genetic drift and determines whether local populations function as independent evolutionary units. Although gene flow has traditionally been seen as a homogenizing force, it can potentially lead to divergence among subpopulations, through the creation and dispersal of unique combinations of alleles (Slatkin 1987).

Among tuco-tucos, the Rio Negro tuco-tuco, *Ctenomys rionegrensis* (Langguth and Abella 1970a), is especially amenable to the study of the forces driving differentiation in this group. Among the reasons for this are: it occupies a relatively small area (50 × 60 km) in Uruguay (there are also three isolated populations in Argentina), where it is restricted to sand dunes; within its small range there is remarkable variation in pelage color, with three primary types recognizable: melanic, agouti (Langguth and Abella 1970a), and dark-backed (Altuna et al. 1985); and in some populations certain pelage types are fixed, whereas in others different combinations of the three types are found (Fig. 1). Earlier work suggested that this chromatic polymorphism is not correlated with soil color or with variation in other environmental characteristics (D'Elía et al. 1998). The existence of two populations monomorphic for the melanic type is particularly interesting because melanic individuals contrast strongly with the sand-dune background. This observation differs from the usual correlation between pelage and substrate colors found among small mammals (Endler 1978), including fossorial species (e.g., Heth et al. 1988; Patton and Smith 1990; Krupa and Geluso 2000), and suggests an adaptative significance, which is presumably a result of selection pressure caused by predation. Pelage color is also known to affect thermoreg-

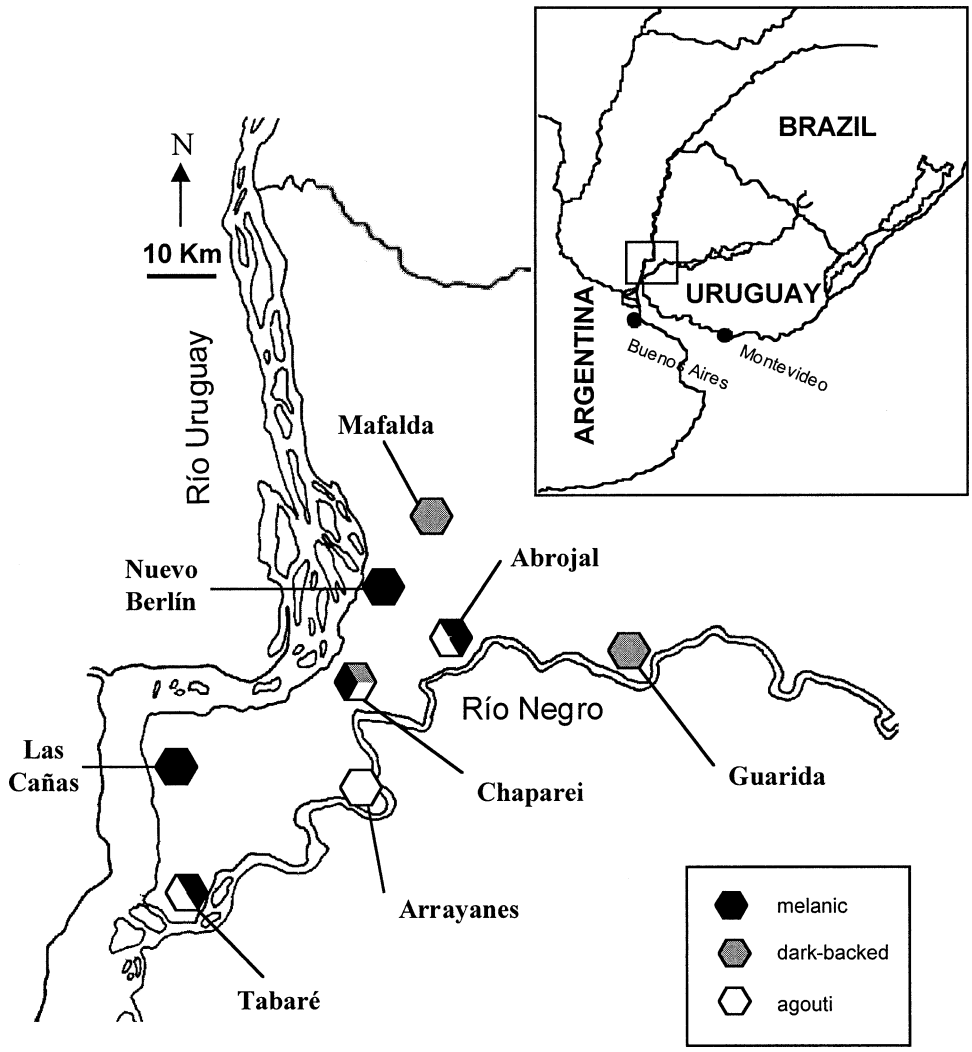

FIG. 1. Map of the geographic distribution of *Ctenomys rionegrensis* in Uruguay. The names of the eight populations sampled are indicated. Geographic coordinates of these localities are: Chaparei (33°06.78′ S, 58°02.17′ W), Abrojal (33°01.75′ S, 57°53.96′ W), La Guarida (33°03.23′ S, 57°43.35′ W), La Tabaré (33°21.41′ S, 58°18.57′ W), Las Cañas (33°11.34′ S, 58°21.33′ W), Arrayanes (33°13.88′ S, 58°01.37′ W), Mafalda (32°52.72′ S, 57°58.30′ W) and Nuevo Berlín (32°58.97′ S, 58°03.85′ W).

ulation and behavior (Endler 1978 and references therein), so more complex selective hypotheses, not necessarily involving matching between pelage and substrate color, can be constructed.

In contrast with other subterranean rodents, *Ctenomys* individuals spend more time above ground associated with foraging and dispersing (Busch et al. 2000), potentially increasing predation risk. It has been documented that different *Ctenomys* species represent up to 18% of the prey items of various owl species (Busch et al. 2000). Predation on *Ctenomys* has also been reported for foxes, grison, opposums, armadillos, and reptilian predators (reviewed in Busch et al. 2000). Although no quantitative studies of predation upon *C. rionegrensis* have been conducted, field observations identified the burrowing owl (*Athene cunicularia*) as a predator of this species, and preliminary surveys of its pellets show that juvenile *C. rionegrensis* are a frequent prey item (E. Lessa, pers. obs.), suggesting a potential role of predation as a selective agent acting on pelage color in this species.

Langguth and Abella (1970b; see also Altuna et al. 1985) assumed a selective pressure imposed by predation against melanic individuals and proposed that melanism in the species might have become locally fixed by genetic drift in spite of its contrast with the background. Subsequently, an allozyme study (D'Elía et al. 1998) estimated moderate to high levels of gene flow among subpopulations of the species with different pelage types ($Nm \cong 6$ to 10). Such estimates are too high to allow for the local fixation of melanism by drift alone and suggest that an undetermined kind of selection (e.g., sexual) might be at play in the fixation of melanism and, more generally, in the maintenance of the chromatic polymorphism (D'Elía et al. 1998).

It is unlikely, however, that current levels of gene flow are as high as suggested by these estimates, given the habitat discontinuity, low vagility, and mean geographic distance between populations (about 30 km). Allozymes have limited resolving power and might be under stabilizing selection, so the obtained values could overestimate current levels of gene

flow (Slatkin 1987). D'Elía et al. (1998) also detected the absence of an equilibrium pattern of isolation by distance, suggesting a recent expansion of geographic range. Genetic estimates of gene flow are based on the expected relationship of *Nm* and F_{ST} at equilibrium, which can be artificially high if such equilibrium has not been reached (Slatkin 1987).

Microsatellite frequencies and mitochondrial DNA sequences are independent—and potentially more sensitive—markers to test the proposed hypotheses concerning the dynamics of differentiation in *C. rionegrensis*. The aims of the present study are to reassess levels of gene flow in the Rio Negro tuco-tuco using microsatellites and mitochondrial DNA and discuss the implications of population structure for the processes of differentiation in the genus *Ctenomys* by comparing and contrasting our results with those of a previous allozyme study. The comparison of patterns in these three datasets provides a strong foundation on which to assess the general dynamics of differentiation in the species, including historical changes in levels of gene flow and isolation.

Materials and Methods

Molecular Methods

Sampling and DNA extraction

Individuals from eight localities, covering the geographic range of *C. rionegrensis* in Uruguay (Fig. 1) and comprising a total of 142 individuals were genotyped with the microsatellite loci described below. Of these 142 animals, 37 were collected at La Tabaré and 15 from each of the remaining localities. A total of 40 individuals, five per locality, were used to study variation at the mitochondrial cytochrome *b* (cyt *b*) gene. The complete sequence of cyt *b* was determined for 37 individuals (see below), and the three remaining sequences, reported by D'Elía et al. (1999), were taken from Genbank (accession numbers: AF119114, AF119104, and AF119103). The samples from the different localities varied substantially in pelage color and included some fixed for each of the pelage colors, as well as some where two or three of the pelage types coexisted (Fig. 1). Liver tissue samples for DNA extraction were collected from freshly sacrificed animals and preserved in 95% ethanol or frozen in liquid nitrogen and transferred to freezers at −70°C. Voucher specimens were prepared and are deposited in the collection of Laboratorio de Evolución, Facultad de Ciencias, Montevideo, Uruguay. Genomic DNA was isolated following a protocol modified from Miller et al. (1988), involving treatment with SDS and digestion with proteinase K, NaCl precipitation of proteins, and subsequent isopropylic alcohol precipitation of DNA.

Polymerase chain reaction amplification and sequencing of mitochondrial cytochrome b

Two overlapping fragments covering the entire cyt *b* gene were amplified by polymerase chain reaction (PCR), using the following primers: MVZ 05 (5′-CGAAGCTTGATATGAAAA-ACCATCGTT-3′; Smith and Patton 1993), -TUCO 06 (5′-GTGAAATGGAATTTTGTCTGA-3′), and TUCO 07 (5′-ATTACAGCAATAGTAATAAT-3′)-TUCO 14A (5′-CCA-ATGTAATTTTTATAC-3′). The PCR amplifications were carried out in a reaction volume of 50 μl, and amplification conditions were the same as described in Lessa and Cook (1998). PCR products were precipitated with 30% polyethylene glycol, washed in 75% ethanol, recovered by vacuum centrifugation, and resuspended in 1× TE buffer. Products were then sequenced with a kit (Fst-RR, 402119; Perkin Elmer, Wellesley, MA), and sequences were run on 4% denaturing polyacrylamide gels using an automated sequencer (ABI 373 or ABI 310). In all cases both heavy and light strands were sequenced. Sequences were assembled based on overlapping regions using Sequence Navigator (Applied Biosystems, Inc., Foster City, CA, ver. 1.0.1) and sequence alignment was performed using the program CLUSTALW (Thompson et al. 1994). The distinct sequences reported in this paper were deposited in GenBank under accession numbers AF538366–AF538377.

Polymerase chain reaction amplification and screening of microsatellites

Eleven microsatellite loci developed from *C. sociabilis* (Soc 1, Soc 2, Soc 6; Lacey 2001) (Soc 5/6; E. Lacey, unpubl. data) and *C. haigi* (Hai 2, Hai 3, Hai 4, Hai 6, Hai 7, Hai 9, Hai 12; Lacey et al. 1999) that proved to be polymorphic in *C. rionegrensis* were surveyed. PCR amplifications were carried out in a reaction volume of 15 μl containing 1 U of AmpliTaq Gold Polymerase (Perkin Elmer), 3 μl of 1/50 total DNA dilution, 1.5 mM $MgCl_2$, 0.2 μM of each primer, and 0.8 mM dNTPs (0.2 mM each). Amplification conditions were as follows: 35 cycles of denaturation at 94°C for 30 sec, annealing at 48°C for 30 sec, and extension at 72°C for 45 sec, preceded by 10 min polymerase activation at 94°C and followed by a final extension at 72°C for 5 min. Seven microliters of PCR product were mixed with 5 μl of stop solution (containing bromophenol blue, xilene cyanol, and 98% deionized formamide) and denatured for 3 min at 94°C before loading. Products were electrophoresed through a 4% denaturing polyacrylamide gel (2000 V, 1–2 h), and visualized by silver nitrate staining according to Sanguinetti et al. (1994). Allelic scoring was done independently by at least two people.

Statistical Analyses

Mitochondrial cytochrome b

Population subdivision was analyzed by assuming the infinite alleles mutation model (Kimura and Crow 1964) and calculating γ_{ST} (Nei 1982) for the whole population. Estimates of global levels of gene flow were calculated from γ_{ST} as: $N_fm \approx 1/2[(1/\gamma_{ST}) - 1]$ (Wright 1951) and also by the private alleles method (Slatkin 1985) with the correction of Barton and Slatkin (1986).

Pairwise estimates of γ_{ST} were calculated using DNASP3 (Rozas and Rozas 1999) to generate pairwise estimates of *M* ($\approx N_fm$). The isolation-by-distance analysis (Slatkin 1993) was performed plotting the log of geographic distances between pairs of populations versus the log of pairwise estimates of *M*. A Mantel test (Mantel 1967) was used to assess the significance of the correlation between these variables,

using 1000 permutations of the matrix as implemented in GENEPOP (version 3.2a; Raymond and Rousset 1995). The relationship between the cyt *b* haplotypes was estimated through a minimum spanning tree obtained using the ARLEQUIN software package (Schneider et al. 2000).

Two population expansion models were evaluated. First, data were fit to a simple model that considers a single ancestral population that undergoes an instantaneous change in size (Wakeley and Hey 1997). This model is described by three parameters, $\theta_{ancestral}$, θ_{final}, and τ (the time at which size change took place in units of N_f generations), which were estimated using the program SITES (Hey and Wakeley 1997). In the second model, demographic expansion parameters were estimated from the mismatch distribution (Rogers and Harpending 1992) by a generalized, nonlinear, least-squares approach, with significance tested using the sum of the squared deviations between the observed and expected mismatch (Schneider and Excoffier 1999).

The neutral expectation of equal ratios of polymorphism to divergence for replacement and silent changes was examined with the McDonald and Kreitman (1991) test using two sequences (Genbank accession numbers AF370695 and AF370696) of the closely related species *Ctenomys mendocinus* from Argentina as an outgroup (Slamovits et al. 2001) in DNASP3 (Rozas and Rozas 1999). Tajima's *D* (Tajima 1989) and Fu's F_s (Fu 1997) were also used to test for a significant excess of low-frequency haplotypes. These analyses were implemented in ARLEQUIN (Schneider et al. 2000).

Microsatellites

Linkage disequilibrium between loci and deviations from Hardy-Weinberg equilibrium (Rousset and Raymond 1995) were tested by a Markov chain method (1000 dememorizations, 50 batches, 1000 iterations) following the algorithm of Guo and Thompson (1992). A Bonferroni correction for multiple tests was applied to give tablewide significance levels of $\alpha = 0.05$.

Population subdivision was analyzed assuming a stepwise mutation model (Ohta and Kimura 1973) and by calculating ρ-statistics (Rousset 1996) for the whole population. Estimates of global levels of gene flow from ρ_{ST} were calculated as $Nm \approx 1/4[(1/\rho_{ST}) - 1]$ (Wright 1951) and by the private alleles method (Slatkin 1985) with the correction of Barton and Slatkin (1986).

Again, pairwise estimates of *M* based on ρ_{ST} were calculated for the isolation-by-distance analysis (Slatkin 1993) and then used for the Mantel test as described above. A Mantel test was also performed to examine correlation between pairwise estimates of gene flow from both datasets ($\log M_{\rho_{ST}}$ and $\log M_{\gamma_{ST}}$). All analyses outlined above were implemented in GENEPOP (Raymond and Rousset 1995).

Maximum-likelihood estimates of a full migration model (allowing unequal population sizes and asymmetric migration rates) based on the coalescent were also obtained by a Markov chain Monte Carlo (MCMC) approach using the program MIGRATE 1.6.5 (Beerli and Felsenstein 2001).

A MCMC approach was used to examine the distinctiveness of subpopulations and the clustering of individual genotypes using the program STRUCTURE (Pritchard et al. 2000). First, a Bayesian test of subdivision, directed at assessing the number of subpopulations solely on the basis of the genotypes, was carried out. Second, individuals were assigned to subpopulations, again without using information concerning their origins.

RESULTS

Mitochondrial DNA and Microsatellite Variation

A total of 15 mitochondrial haplotypes were identified in the sample (Table 1). Variation was observed at 18 positions (17 transitions and one transversion), of which five were replacement changes. Following exclusion of ambiguous sites, 1121 bp of the cyt *b* sequence were usable for subsequent analysis.

All 11 of the microsatellite loci surveyed were polymorphic (Table 2). The total numbers of alleles per locus ranged from 6 (Hai 7, Hai 9, Soc 2) to 14 (Hai 4), with an average of 8.3 alleles. Allelic diversity (*A*), calculated as the mean number of alleles detected per locus varied from 1 ($\pm$0.0; Nuevo Berlín) to 4.7 ($\pm$1.9; Arrayanes). Values of expected heterozygosity (H_e) averaged across loci ranged from 0 (Nuevo Berlín) to 0.63 (Chaparei, Arrayanes). Microsatellite allele frequencies are shown in the Appendix.

Exact tests of genotypic linkage disequilibrium (either global or for subpopulations) yielded no significant values ($P > 0.05$), suggesting that loci are independent. Significant positive departures from Hardy-Weinberg equilibrium were found in a few loci in some populations (Hai 2: Chaparei, La Tabaré; Hai 7: Mafalda; Hai 9: La Tabaré; Hai 6: Abrojal; Soc 2: La Guarida). When a global test across loci and across populations was performed, the null hypothesis of equilibrium was rejected ($P < 0.001$).

Geographic Variation

Except for Soci 2, population-specific alleles were detected for all microsatellite loci. Nuevo Berlín, one of the two entirely melanic populations, was monomorphic for all 11 loci. Alleles fixed in the Nuevo Berlín population were, in all cases, the most frequent or the second most frequent ones across all other populations (see Appendix). Local fixation of particular alleles for some loci was observed in other cases (Hai 2 in El Abrojal, Hai 12–Soc 1 in La Guarida, Hai 12 in La Tabaré, Hai 9–Hai 12 in Las Cañas) and always involved alleles with high overall frequencies in the total sample.

Using a Bayesian MCMC approach, the presence of eight populations in the sample was inferred solely on the basis of multilocus microsatellite genotypes. Considering the range of six to 10 potential populations, for example, the posterior probability of the data coming from eight populations is $>$0.9999, whereas the corresponding probabilities for seven or nine populations are 10^{-38} and 4.5×10^{-5}, respectively. Values further away from eight decrease dramatically. Assuming eight populations, genotypes have average ancestries from their corresponding populations ranging from 0.895 to 0.987. Individuals were correctly assigned into the population they belonged to in 100% of cases. Only two individuals

TABLE 1. Polymorphic sites (excluding ambiguous sites) at the mitochondrial cytochrome *b* gene for *Ctenomys rionegrensis* ($N = 40$). Nucleotides are numbered from 1 to 1140. The distribution of each haplotype, by locality (CH, Chaparei; A, Abrojal; G, La Guarida; T, La Tabaré; LC, Las Cañas; AR, Arrayanes; M, Mafalda; and B, Nuevo Berlín), and the total number of individuals per haplotype are shown.

	No. site																	Distribution of haplotypes by locality								
No. haplotype	0 5 2	0 6 7	0 0 9	0 1 0	0 2 0	0 3 5	0 4 0	0 4 8	0 5 1	0 6 6	0 9 6	0 6 6	0 8 7	0 8 9	0 2 1	1 2 0	1 5 3	1 0 8	1 0 7	1 0 9	1 0 2	CH	A	G	T	LC	AR	M	B	Total
1 (AF119103)	T	G	T	T	T	A	A	A	C	A	C	T	A	G	C	C	T	T	T	T	T	—	—	—	—	5	—	—	—	5
2 (AF538366)	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	—	—	—	2	—	—	—	—	2
3 (AF538377)	.	.	.	.	.	.	G	.	.	.	.	.	.	A	.	T	.	.	.	.	.	—	—	—	3	—	—	—	—	3
4 (AF119104)	.	.	.	.	.	G	G	.	.	.	.	.	.	.	.	T	.	.	.	.	.	—	—	—	—	1	—	—	—	1
5 (AF538367)	.	.	.	.	.	G	G	.	.	T	.	.	.	.	.	T	.	.	.	.	.	—	—	—	—	2	—	—	—	2
6 (AF538368)	.	.	.	.	.	G	G	.	T	.	.	.	T	.	.	T	.	.	.	.	.	—	—	—	—	1	—	—	—	1
7 (AF538369)	.	.	.	.	.	.	G	.	T	.	.	.	.	.	.	T	.	.	.	.	.	—	—	—	—	1	—	—	—	1
8 (AF538370)	.	.	.	.	.	.	G	.	.	.	.	G	.	.	T	T	.	.	.	.	.	—	—	—	—	—	1	—	—	1
9 (AF538371)	.	.	.	.	C	.	.	G	.	.	.	.	.	.	.	T	.	.	.	.	.	—	—	—	—	—	1	—	—	1
10 (AF538372)	.	.	.	.	.	.	.	G	.	G	.	.	.	.	.	T	.	.	.	.	.	—	—	—	—	—	1	—	—	1
11 (AF119114)	.	.	.	C	.	.	.	G	.	.	.	.	.	.	.	T	C	.	.	.	.	—	5	—	—	—	—	—	—	5
12 (AF538373)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	T	.	.	.	.	.	—	—	5	—	—	—	2	5	12
13 (AF538374)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	T	C	.	.	.	.	3	—	—	—	—	—	—	—	3
14 (AF538375)	C	.	.	.	.	.	.	G	.	.	.	C	.	.	.	T	.	.	C	.	.	1	—	—	—	—	—	—	—	1
15 (AF538376)	C	.	.	.	C	.	.	G	.	.	.	C	.	.	.	T	.	.	C	.	.	1	—	—	—	—	—	—	—	1

were suggested to have mixed ancestry (with assignment probability to the correct population always being higher). Just a few individuals had assignment probabilities between 0.85 and 0.95, with the rest above 0.95.

The minimum spanning network of cyt *b* haplotypes (Fig. 2) shows a most frequent haplotype, from which each of the others differs by just one or a few changes. This frequent haplotype is the only one shared among populations. In contrast, several haplotypes have a frequency of $1/n$.

In sum, the above results show that the species is strongly structured geographically, with subpopulations constituting distinct genetic entities, but sharing a recent common ancestor.

Estimates of Gene Flow

The mean number of migrants per generation (*Nm*) calculated from global ρ_{ST}- and γ_{ST}-values was 0.45 and 0.55, respectively. Global levels of gene flow estimated by Slatkin's private alleles method were $Nm = 0.373$ for the microsatellite data and $N_f m = 0.296$ for the mitochondrial data. Table 3 shows pairwise estimates of gene flow. For the microsatellite data, *Nm*-values ranged from 0.062 to 2.69. For the cyt *b* sequence data, values ranged from 0.0 to 4.0.

No pattern of isolation by distance is evident when log of *Nm* (calculated either from ρ_{ST} or γ_{ST}) is plotted against log of geographic distance between pairs of populations (Fig. 3). Mantel tests show no significant correlation between pairwise estimates of log *Nm* (based on ρ_{ST} or γ_{ST}) and log geographic distances or between both matrices of pairwise estimates of log *Nm* ($P > 0.05$). For species with restricted dispersal, the absence of a pattern of isolation by distance is an indicator of nonequilibrium, suggesting that the species has recently colonized its present distribution (Slatkin 1993).

Table 4 shows maximum-likelihood estimates of pairwise migration rates (expressed as *Nm*) and of θ ($=4N\mu$), obtained for a full migration model. In all cases, migration rates were below $Nm = 1$, ranging from $Nm = 2.423 \times 10^{-15}$ to 0.106. Estimates of θ were similar for the eight populations, ranging from 0.079 to 0.171.

Population Expansion

Using the Wakeley and Hey (1997) size-change model, estimates of the relative sizes of the ancestral and descendent populations, as well as the time the size change took place, were: $\theta_{ancestral} = 0$, $\theta_{final} = 11.06$, and $\tau = 2.513$ (in units of N_f generations). The same parameters estimated by the Rogers and Harpending (1992) model of population expansion were: $\theta_{ancestral} = 0$ (95% CI = 0.000–1.357), $\theta_{final} = 83.75$ (95% CI = 6.303–7770.0), and $\tau = 2.403$ mutational units (95% CI = 1.247–3.188). Both models had poor fit but were consistent in suggesting population expansion.

A significant excess of low-frequency haplotypes is shown by Fu's test ($F_s = -26.85$, $P < 0.00001$) and, although not significant, is also suggested by the more conservative Tajima's test ($D = -1.539$, $P > 0.0549$). One possible explanation is that there is a real departure from the neutral mutation model, with deleterious mutants being maintained at low frequencies. However, the same pattern can be obtained if the population has recently expanded from a smaller size

TABLE 2. Summary of microsatellite variation in *Ctenomys rionegrensis* populations. The number of alleles per local (*N*), observed (H_o) and expected heterozygosities (H_e), and the result of Hardy-Weinberg analyses (*P*-values for each locus and population) are reported.

Population	Chaparei		Abrojal		Guarida		Tabaré		Las Cañas		Arrayanes		Mafalda		N. Berlín		Global		Hardy-Weinberg
Locus	*N*	H_o H_e	*N*	H_o H_e	*N*	H_o H_e	*N*	H_o H_e	*N*	H_o H_e	*N*	H_o H_e	*N*	H_o H_e	*N*	H_o H_e	*N*	H_o H_e	*P*-value
Hai2	4	0.40	1	0	2	0.13	4	0.51	3	0.33	3	0.73	3	0.53	1	0	2.63	0.33	0.0139
		0.68		0		0.24		0.70		0.54		0.55		0.48		0		0.40	
Hai3	7	0.66	4	0.53	2	0.13	2	0.41	2	0.73	4	0.33	5	0.67	1	0	3.38	0.43	0.1420
		0.84		0.57		0.33		0.50		0.51		0.36		0.61		0		0.47	
Hai7	7	0.73	4	0.53	2	0.07	3	0.30	2	0.33	3	0.80	3	0.33	1	0	3.13	0.39	0.3673
		0.84		0.61		0.07		0.30		0.48		0.60		0.48		0		0.42	
Hai9	3	0.80	3	0.60	2	0.27	4	0.32	1	0	2	0.40	3	0.53	1	0	2.38	0.37	0.0377
		0.60		0.62		0.50		0.46		0		0.46		0.58		0		0.40	
Hai12	2	0.33	1	0	1	0	1	0	1	0	5	0.73	4	0.60	1	0	2	0.21	0.3358
		0.43		0		0		0		0		0.72		0.53		0		0.21	
Soc1	4	0.53	4	0.73	1	0	2	0.19	2	0.53	6	0.67	3	0.53	1	0	2.88	0.40	0.5343
		0.73		0.71		0		0.03		0.50		0.82		0.58		0		0.42	
Soci5/6	4	0.60	2	0.53	2	0.27	4	0.51	2	0.33	6	0.80	5	0.60	1	0	3.25	0.46	0.6240
		0.74		0.46		0.33		0.63		0.29		0.74		0.65		0		0.48	
Hai6	3	0.47	4	0.33	2	0.20	4	0.57	4	0.47	5	0.40	6	0.67	1	0	3.63	0.39	0.0291
		0.43		0.54		0.28		0.67		0.41		0.58		0.59		0		0.44	
Hai4	3	0.33	7	0.80	4	0.27	5	0.76	4	0.80	9	0.80	4	0.20	1	0	4.63	0.50	0.7299
		0.57		0.84		0.31		0.75		0.64		0.88		0.19		0		0.52	
Soc2	5	0.60	3	0.20	3	0.53	4	0.54	2	0.40	5	0.93	5	0.67	1	0	3.5	0.48	0.3204
		0.62		0.19		0.54		0.52		0.50		0.75		0.73		0		0.48	
Soc6	2	0.47	2	0.40	2	0.20	4	0.65	2	0.33	4	0.60	2	0.60	1	0	2.38	0.41	0.9866
		0.48		0.50		0.19		0.69		0.43		0.53		0.48		0		0.41	
Global	4	0.54	3.18	0.42	2.09	0.19	3.36	0.43	2.27	0.39	4.73	0.65	3.91	0.54	1	0	8.30	—	
		0.63		0.46		0.25		0.48		0.39		0.64		0.54		0			
Hardy-Weinberg *P*-value	0.0735		0.5049		0.0587		0.0224		0.5733		0.4100		0.872		—				0.0007

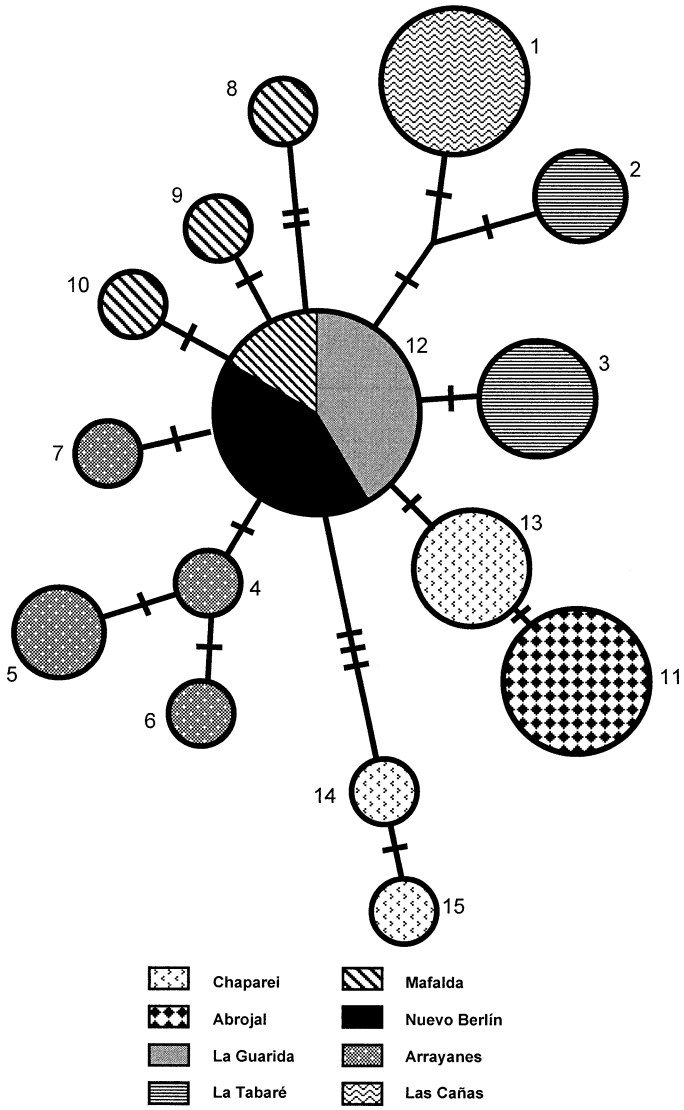

FIG. 2. Minimum spanning tree of 15 cytochrome *b* gene haplotypes. Circle areas are proportional to haplotype frequencies. Haplotype numbers correspond to those of Table 1. Shading indicates populations.

TABLE 3. Pairwise estimates of gene flow (*M*) based on γ_{ST} (upper half matrix) and ρ_{ST} (lower half matrix) for cytochrome *b* gene sequences and microsatellites, respectively. Populations: 1, Chaparei; 2, Abrojal; 3; La Guarida; 4, La Tabaré; 5, Las Cañas; 6, Arrayanes; 7, Mafalda; 8, Nuevo Berlín.

Population	1	2	3	4	5	6	7	8
1		0.67	1.27	1.18	0.39	1.05	1.69	1.27
2	1.35		0.00	0.27	0.00	0.25	0.30	0.00
3	1.99	1.42		1.06	0.00	1.82	4.00	—
4	0.73	0.46	0.62		0.38	0.92	1.62	1.06
5	1.51	0.40	0.38	0.41		0.25	0.30	0.00
6	1.48	1.57	1.44	2.69	0.54		1.31	0.82
7	0.27	0.33	0.20	0.38	0.12	0.90		4.00
8	0.16	0.15	0.07	0.43	0.06	0.65	0.25	

and remains away from an equilibrium between mutation and drift (Tajima 1989; Fu 1997).

In the comparison of *C. rionegrensis* and *C. mendocinus*, seven replacement and 17 silent sites were polymorphic, whereas one replacement and 18 silent differences were fixed. This results in a statistically significant McDonald-Kreitman test ($G = 4.508$, $P < 0.03$), also indicating a departure from strict neutrality.

In sum, the data are consistent with a demographic expansion, from a more restricted distribution to the current range. For cyt *b*, selection against slightly deleterious mutations may be invoked in place of or in addition to demographic change, to account for the data.

DISCUSSION

Absence of Migration-Drift Equilibrium and Population Expansion

Taken as a whole, the results presented here strongly support the hypothesis of a recent range expansion for *C. rionegrensis*. Three separate molecular datasets, the microsatellite and mitochondrial DNA data presented here and the allozyme data presented by D'Elía et al. (1998), are consistent in this respect.

The absence of an isolation-by-distance pattern, as shown by the lack of significant correlation between pairwise estimates of *Nm* and geographic distance (Fig. 3), suggests that the species is not at an equilibrium between gene flow and genetic drift. In stepping-stone models of migration (adequate to describe population structure of species with geographically limited dispersal), the log of expected values of *M* at equilibrium plotted against log of distance shows an approximately linear relationship, with *M* decreasing as the distance separating populations increases (Slatkin 1993). As Slatkin pointed out, the absence of such a pattern suggests that the species is far from an equilibrium between gene flow and genetic drift and may have only recently colonized parts of the area it currently occupies.

The equilibrium between the loss of alleles due to drift and their replacement by gene flow is reached when the migration regime established after the population expansion remains stable across the geographic range for a long enough time. However, if populations become completely isolated almost immediately after expansion, then that equilibrium may never be reached and the isolation-by-distance pattern will not evolve. Instead, the signature of the historical connection (e.g., high estimates of *M*) will be strong initially, but decay with time for all pairs of populations regardless of geographic proximity.

Although the mitochondrial DNA data poorly fit models of population expansion, the estimates of increase in population size and time obtained are in accordance with a recent population expansion. The poor fit to the Wakeley and Hey size-change model can be explained by the high variances associated with using just one nonrecombining locus for this model (Wakeley and Hey 1997) and by the fact that data from eight populations are treated as representing a single population. Either recombination or the use of multiple loci should improve the quality of parameter estimates (Pluzhnikov and Donnelly 1996). Nonoverlapping 95% confidence

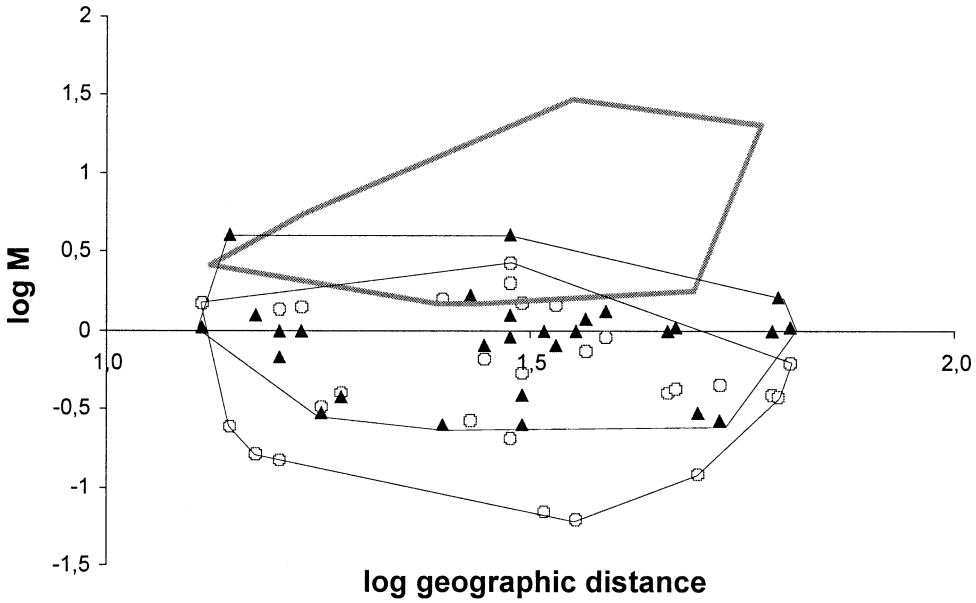

FIG. 3. Relationships between pairwise geographic distances and estimates of gene flow (M) based on F_{ST}, γ_{ST}, and ρ_{ST} for allozymes, cytochrome *b* gene sequences, and microsatellites, respectively. Circles and triangles indicate microsatellite and cytochrome *b* estimates, respectively. The thick gray line shows the range of previous allozyme estimates.

intervals of the estimates of current and ancestral θ were obtained for the Rogers and Harpending population expansion model. Both models provide an approximate idea of the magnitude of population size change and indicate that it must have been at least 10-fold. To transform the estimates of τ into time (in years or generations) would require additional information about neutral mutation rates, current effective population size, or generation time that are not available at this point.

Additional evidence of a population expansion comes from Tajima's D and Fu's F_s. For neutral markers, and assuming a constant mutation rate, significantly negative D- or F_s-values are strong evidence of a population expansion because they indicate an excess of low-frequency variants, as expected after such an event. The hypothesis of recent population expansion is also supported by a starlike topology of haplotype relationships (Slatkin and Hudson 1991), as found in the minimum spanning tree (Fig. 2).

Although the cyt *b* gene has been widely assumed to be neutral and is used extensively as a genetic marker, positive selection cannot be ruled out (Whittam et al. 1986; Harrison 1989; Ballard and Kreitman 1995). In fact, several studies have detected departures from the neutral model in the mitochondrial genome (Nachman et al. 1994, 1996; Nachman 1998; Rand and Kann 1998). Both a selective sweep and selection against slightly deleterious mutations can produce a pattern of haplotype diversity similar to that produced by a population expansion; therefore, these alternatives cannot be discriminated with statistics such as Tajima's D or Fu's F_s. The McDonald and Kreitman test, however, shows that a selective explanation for the variation at the cyt *b* gene is compatible with the data. Selection on that locus and demographic expansion are, in sum, two nonmutually exclusive hypotheses compatible with the data.

Neigel et al. (1991) suggested that the most geographically widespread haplotypes should be the oldest and ancestral ones, under a limited gene flow model. Conversely, haplotypes restricted to single locations should be of more recent origin. Following this line of reasoning, the area comprised of Nuevo Berlín, Mafalda, and La Guarida could be ancestral, given that they share the most frequent mitochondrial haplotype.

Gene Flow Estimates

Overall, the three kinds of markers show substantially different estimates of gene flow that must be explained. Levels of gene flow estimated from the cyt *b* gene are markedly lower than those suggested by allozymes. In turn, estimates from the microsatellite data are even lower. The scatter of pairwise estimates (calculated from ρ_{ST} or γ_{ST}) is such that those resulting from mitochondrial data are intermediate and partially overlap those from allozymes and microsatellites (Fig. 3). Before proposing a comprehensive hypothesis to account for these contrasts, the potential effects of four factors on estimates of gene flow are considered.

First, we explore differences in male and female contributions to gene flow. Sex-biased migration may occur in tucotucos (Malizia and Busch 1991). However, it clearly cannot account for the observed pattern, as gene flow estimates from the two sets of autosomal markers are more different from each other than either is to those derived from the maternally inherited mitochondrial cyt *b*.

Second, we consider differences in mutation rates among markers. At equilibrium, estimates of gene flow based on F_{ST} or analogous statistics, such as the ones used here, are approximately independent from neutral mutation rates, provided that these rates are low. Although direct estimates of neutral mutation rates are not available, it is reasonable to assume that they span several orders of magnitude, from low

TABLE 4. Maximum likelihood estimates of gene flow and population sizes for microsatellite data.

Population	$4N\mu$	*Nm* (x, receiving population)							
		1, x	2, x	3, x	4, x	5, x	6, x	7, x	8, x
1: Chaparei	0.127	—	2.975×10^{-15}	0.059	0.022	0.010	0.106	0.018	0.005
2: Abrojal	0.115	0.011	—	0.012	0.012	0.029	2.700×10^{-15}	0.028	0.015
3: La Guarida	0.109	0.008	0.012	—	0.012	0.009	0.021	0.068	0.020
4: La Tabaré	0.171	0.068	0.010	0.078	—	0.030	5.575×10^{-13}	0.010	0.092
5: Las Cañas	0.094	0.013	0.020	0.0164	0.021	—	2.423×10^{-15}	0.007	0.010
6: Arrayanes	0.079	6.45×10^{-12}	0.022	0.024	0.009	0.012	—	0.009	0.016
7: Mafalda	0.102	0.021	0.004	0.005	0.019	0.029	0.035	—	0.029
8: Nuevo Berlín	0.115	2.318×10^{-14}	0.063	0.037	0.009	0.037	0.013	0.035	—

for allozymes ($\sim 10^{-6}$/locus/generation; Nei 1987) to very high for microsatellites (10^{-5}–10^{-2}/locus/generation; Hancock 1999). The unusually high (and complex) mutation process in microsatellites is taken into account, at least in part, by the use of ρ_{ST}, instead of F_{ST}, to estimate gene flow from those loci. More importantly, estimates of gene flow from rare alleles for each of the three datasets are concordant with those from F_{ST} or its analogs. These estimates are less sensitive to high mutation rates or selection (Slatkin 1985; Hedrick 1999). Overall, it is clear that the differences in gene flow estimates cannot simply be the result of the differential impact of mutation.

Third, we examine the impact of nonequilibrium on genetic estimates of gene flow. The absence of equilibrium has important implications for the estimation of population genetic parameters. Wright's (1951) approximate equation is based on the island model of population structure (although it also applies to stepping stone models; Slatkin and Barton 1989) and assumes that populations are at an equilibrium between gene flow and genetic drift. When these assumptions are violated, genetic estimates of gene flow are not reliable. Gene flow estimates are probably greatly affected by the proposed recent expansion and likely exhibit an important historical component, rather than reflecting current levels of gene flow (Slatkin 1993). Moreover, markers with different effective population sizes such as mitochondrial and nuclear DNA (the mitochondrial genome has an effective population size one-quarter that of autosomal loci) are unequally affected and return to equilibrium at different rates (Fay and Wu 1999).

Finally, we consider the impact of nonneutral variation on genetic estimates of gene flow. It has long been recognized that positive selection can distort population genetic parameter estimates. For instance, balanced allozyme polymorphisms across the species distribution will result in elevated estimates of *Nm* (Slatkin 1987). The fact that rare allele estimates, which are less sensitive to selection, are concordant with those based on F_{ST} or its analogs is somewhat reassuring. However, the maintenance of allozyme polymorphisms in Nuevo Berlín, where all 11 microsatellite markers are monomorphic, suggests that the selective maintenance of at least some allozyme polymorphisms is possible. More generally, the similarities between subpopulations are higher as one moves from noncoding microsatellites to cyt *b* (in which most polymorphisms are silent) to allozymes (in which amino acid changes are required for the detection of variants). The opportunities for positive selection, although not known, probably rank similarly.

In sum, it appears that each of the four factors considered above may contribute to, but none can by itself account for, the observed pattern. All the information taken together suggests the following scenario. *Ctenomys rionegrensis* expanded into its current geographic range from a more restricted area at a relatively recent time in the past. This is suggested by the lack of migration-drift equilibrium in pairwise analyses of gene flow and geographic distance found for all datasets and by coalescent-based estimates of demographic expansion for the cyt *b* data. A more restricted, but continuous, distribution of suitable habitat in the past might be invoked to explain the sudden population expansion, given the extremely patchy nature of sandy soils that this species inhabits. Pleistocene/Holocene marine transgressions are known to have affected the geographic region that comprises the present distribution of *C. rionegrensis* (Alonso 1978; Sprechmann 1978). Unfortunately, not enough detail is known about these events to make a compelling case in this respect.

Since the expansion, subpopulations have differentiated, essentially in isolation, under the influence of genetic drift and mutation. The moderate to high estimates of gene flow from allozymes and mitochondrial DNA are inflated by the impact of the recent range expansion. For the microsatellite data, high mutation rates contributed to more rapid differentiation and correspondingly lower estimates of gene flow between subpopulations.

Positive selection may, at least in the case of allozymes, contribute to the retention of genetic similarities between subpopulations and, consequently, to elevated estimates of gene flow (Karl and Avise 1992; Pogson et al. 1995; Schmidt and Rand 2001). Selection may also explain the presence of allozyme polymorphism in the Nuevo Berlín population, which is invariant for microsatellites. A departure from strict neutrality may also contribute to the retention of similarities in cyt *b* across populations.

Considering all of these factors, estimates of gene flow from microsatellites are likely the most reliable, especially because they are an average across 11 loci, and may come close to estimating current levels of migration. Microsatellites are likely to underestimate genetic divergence between populations, due to their high mutation rates, when *F*-statistics are employed and especially when migration is low (Lehman et al. 1996; Hedrick 1999). A simulation study provides further caution about the interpretation of population structure based on this kind of marker when gene flow is reduced (Balloux et al. 2000). This would suggest even lower levels of gene flow between populations than measured from ρ_{ST}

for the microsatellite data. This is confirmed by the maximum-likelihood estimates, the highest of which is $Nm = 0.106$.

Slatkin (1993) proposed that low values of M and the lack of a pattern of isolation by distance indicate that there is essentially no current gene flow. Similarly, Hutchison and Templeton (1999) argue that the lack of regional equilibrium (in their case using F_{ST} rather than M), with a wide scatter over all degrees of geographic distances is a clear indication of a region occupied by highly isolated populations in which genetic drift has had a prominent role.

It would appear that subpopulations of *C. rionegrensis* are likely connected by current levels of gene flow much lower than previously believed, perhaps to the extreme of being completely isolated. Multilocus genotypes allow for correct assignment of all individuals to their corresponding populations, even without using prior information about the actual number of existing populations. This indicates that population subdivision is very strong, with subpopulations behaving as discrete units and their allelic frequencies varying independently.

Chromatic Polymorphism

As mentioned above, the hypothesis of local fixation of melanism by drift alone was previously rejected on the basis of high levels of gene flow estimated from allozyme data (D'Elía et al. 1998). However, these authors cautioned against overinterpretation of the data, due to the potential role of positive selection and violation of the assumption of equilibrium between migration and drift. In light of the data presented here, the high levels of gene flow estimated in that study appear to be less reflective of current migration than the much lower estimates from microsatellites.

If current levels of gene flow are very low, the local fixation of alleles, including those responsible for pelage color polymorphism, by drift alone is possible. Small population size would further increase the power of genetic drift in these isolated populations. The two exclusively melanic populations, Nuevo Berlín and Las Cañas (together with La Guarida), have the lowest mean numbers of alleles per microsatellite locus, which is probably reflective of their small population size.

High levels of gene flow, such as those suggested by allozymes, would preclude the local fixation of alternative pelage colors by drift alone. Importantly, however, low levels of gene flow, such as those indicated by our data, only indicate that random fixation is possible, but do not really allow us to rule out selection as a process affecting pelage color variation. Classical analyses of predation pressure by owls on deer mice, for example, have yielded selection coefficients above 0.2 (Dice 1947), thus implying that, in the absence of gene flow, extremely small population sizes are required for drift to overwhelm selection. In the case of *C. rionegrensis*, such estimates of selection coefficients are lacking.

An examination of the pattern of inheritance of pelage colors and the reproductive interaction of the different pelage forms has just begun. Ultimately, only the direct study of the genes responsible for the chromatic polymorphism will resolve the questions about its evolution and maintenance.

Acknowledgments

We thank T. B. Smith and two anonymous reviewers for helpful comments on an earlier version of this article. We are grateful to Comisión Sectorial de Investigación Científica, Universidad de la República and the Consejo Nacional de Investigaciones Científicas y Técnicas (Uruguay) for financial support and to E. Lacey (Museum of Vertebrate Zoology, University of California at Berkeley) for generously providing primers before publication. EPL is indebted to the Guggenheim Foundation and to J. Wakeley (Harvard University) for his expert guidance in the study of population genetics.

Literature Cited

Alonso, C. 1978. La fauna de moluscos del yacimiento de Playa Pascual con referencia a otros yacimientos estuaricos y marinos del cuaternario de Uruguay. Com. Soc. Malacol. Uruguay 4: 365–383.

Altuna, C., M. Ubilla, and E. P. Lessa. 1985. Estado actual del conocimiento de *Ctenomys rionegrensis* Langguth y Abella, 1970 (Rodentia: Octodontidae). Actas Jor. Zool. Uruguay 1:8–9.

Ballard, J. W. O., and M. Kreitman. 1995. Is mitochondrial DNA a strictly neutral marker? Trends Ecol. Evol. 10:485–488.

Balloux, F., H. Brünner, N. Lugon-Moulin, J. Hausser, and J. Goudet. 2000. Microsatellites can be misleading: an empirical and simulation study. Evolution 54:1414–1422.

Barton, N. H., and M. Slatkin. 1986. A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. Heredity 56:409–415.

Beerli, P., and J. Felsenstein. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in *n* subpopulations by using a coalescent approach. Proc. Natl. Acad. Sci. 98:4563–4568.

Busch, C., C. D. Antinuchi, J. C. del Valle, M. J. Kittlein, A. I. Malicia, A. I. Vasallo, and R. R. Zenuto. 2000. Population ecology of subterranean rodents. Pp. 183–226 *in* E. A. Lacey, J. L. Patton, and G. N. Cameron, eds. Life underground: the biology of subterranean rodents. Univ. of Chicago Press, Chicago, IL.

Bush, G. 1994. Sympatric speciation in animals: new wine in old bottles. Trends Ecol. Evol. 9:285–288.

Coyne, J. A. 1992. Genetics and speciation. Nature 355:511–515.

D'Elía, G., E. P. Lessa, and J. A. Cook. 1998. Geographic structure, gene flow and maintenance of melanism in *Ctenomys rionegrensis* (Rodentia: Octodontidae). Z. Säugetierkde. 63:285–296.

———. 1999. Molecular phylogeny of tuco-tucos, genus *Ctenomys* (Rodentia: Octodontidae): evaluation of the mendocinus species group and the evolution of asymmetric sperm. J. Mammal. Evol. 6:19–38.

Dice, L. R. 1947. Effectiveness of selection by owls of deer-mice (*Peromyscus maniculatus*) which contrast in color with their background. Contributions from the Laboratory of Vertebrate Biology, Univ. of Michigan, Ann Arbor, MI.

Endler, J. A. 1978. A predator's view of animal color patterns. Pp. 319–364 *in* M. K. Hecht, W. C. Steere, and B. Wallace, eds. Evolutionary biology. Vol. 11. Plenum Press, New York.

Fay, J. C., and C. I. Wu. 1999. A human population bottleneck can account for the discordance between patterns of mitochondrial versus nuclear DNA variation. Mol. Biol. Evol. 16:1003–1005.

Fu, Y. X. 1997. Statistical test of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915–925.

Guo, S. W., and E. A. Thompson. 1992. Performing the exact test for Hardy-Weinberg proportion for multiple alleles. Biometrics 48:361–372.

Hancock, J. M. 1999. Microsatellites and other simple sequences: genomic context and mutational mechanism. Pp. 1–9 *in* D. B. Goldstein and C. Schlötterer, eds. Microsatellites: evolution and applications. Oxford Univ. Press, New York.

Harrison, R. G. 1989. Animal mitochondrial DNA as a genetic

marker in population and evolutionary biology. Trends Ecol. Evol. 4:6–11.
Hedrick, P. W. 1999. Perspective: Highly variable loci and their interpretation in evolution and conservation. Evolution 53: 313–318.
Heth, G., A. Beiles, and E. Nevo. 1988. Adaptative variation of pelage color within and between species of the subterranean mole rat (*Spalax ehrenbergi*) in Israel. Oecologia 74:617–622.
Hey, J., and J. Wakeley. 1997. A coalescent estimator of the population recombination rate. Genetics 145:833–846.
Hutchison, D. W., and A. R. Templeton. 1999. Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. Evolution 53:1898–1914.
Karl, S. A., and J. C. Avise. 1992. Balancing selection at allozyme loci in oysters: implications from nuclear RFLPs. Science 256: 100–102.
Kimura, M., and J. F. Crow. 1964. The number of alleles that can be maintained in a finite population. Genetics 49:725–738.
Kondrashov, A. S., L. Y. Lampolski, and S. A. Shabalina. 1998. On the sympatric origin of species by means of natural selection. Pp. 90–98 *in* D. J. Howard and S. H. Berlocher, eds. Endless forms: species and speciation. Oxford Univ. Press, Oxford, U.K.
Krupa, J. J., and K. N. Geluso. 2000. Matching the color of excavated soil: cryptic coloration in the plains pocket gopher (*Geomys bursarius*). J. Mammal. 81:86–96.
Lacey, E. A. 2001. Microsatellite variation in solitary and social tuco-tucos: molecular properties and population dynamics. Heredity 86:628–637.
Lacey, E. A., J. E. Maldonado, J. P. Clabaugh, and M. D. Matocq. 1999. Interspecific variation in microsatellites isolated from tuco-tucos (Rodentia: Ctenomyidae). Mol. Ecol. 8:1754–1756.
Lacey, E. A, J. L. Patton, and G. N. Cameron, eds. 2000. Life underground: the biology of subterranean rodents. Univ. of Chicago Press, Chicago, IL.
Langguth, A., and A. Abella. 1970a. Las especies uruguayas del género *Ctenomys*. Com. Zool. Mus. Hist. Nat. Montevideo 10: 1–27.
———. 1970b. Sobre una población de tuco-tucos melánicos (Rodentia: Octodontidae). Acta Zool. Lilloana 28:101–108.
Lehman, T. W., A. Hawley, L. Kamau, D. Fontenille, F. Simard, and F. H. Collins. 1996. Genetic differentiation of *Anopheles gambiae* populations from East and West Africa: comparison of microsatellite and allozyme loci. Heredity 77:192–200.
Lessa, E. P., and J. A. Cook. 1998. The molecular phylogenetics of tuco-tucos (genus *Ctenomys*, Rodentia: Octodontidae) suggests an early burst of speciation. Mol. Phylogenet. Evol. 9: 88–99.
Malizia, A. I., and C. Busch. 1991. Reproductive parameters and growth in the fossorial rodent *Ctenomys talarum* (Rodentia: Octodontidae). Mammalia 55:293–305.
Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27:209–220.
McDonald, J. H., and M. Kreitman. 1991. Adaptative protein evolution at the Adh locus in *Drosophila*. Nature 351:652–654.
Miller, S. A., D. D. Dikes, and H. F. Polesky. 1988. A simple salting out procedure for extracting DNA for human nucleated cells. Nucleic Acids Res. 16:215.
Nachman, M. W. 1998. Deleterious mutations in animal mitochondrial DNA. Genetica 102/103:61–69.
Nachman, M. W., S. N. Boyer, and C. F. Aquadro. 1994. Nonneutral evolution at the mitochondrial NADH dehydrogenase subunit 3 gene in mice. Proc. Natl. Acad. Sci. USA 91:6364–6368.
Nachman, M. W., W. M. Brown, M. Stoneking, and C. F. Aquadro. 1996. Nonneutral mitochondrial DNA variation in humans and chimpanzees. Genetics 142:953–963.
Nei, M. 1982. Evolution of human races at the gene level. Pp. 167–181 *in* B. Bonne-Tamir, T. Cohen, and R. M. Goodman, eds. Human genetics. Part A: the unfolding genome. Alan R. Liss, New York.
———. 1987. Molecular evolutionary genetics. Columbia Univ. Press, New York.
Neigel, J. E., R. M. Ball Jr., and J. C. Avise. 1991. Estimation of single generation dispersal migration distances from geographic variation in animal mitochondrial DNA. Evolution 45:423–432.
Ohta, T., and M. Kimura. 1973. A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. Genet. Res. 22:201–204.
Orr, M. R., and T. B. Smith. 1998. Ecology and speciation. Trends Ecol. Evol. 13:502–506.
Patton, J. L., and M. F. Smith. 1990. The evolutionary dynamics of the pocket gopher *Thomomys bottae*, with emphasis on California populations. Univ. Calif. Publ. Zool. 123:1–161.
Pluzhnikov, A., and P. Donnelly. 1996. Optimal sequencing strategies for surveying molecular genetic diversity. Genetics 144: 1247–1262.
Pogson, G. H., K. A. Mesa, and R. G. Boutilier. 1995. Genetic population structure and gene flow in the Atlantic cod *Gadus morhua*: a comparison of allozyme and nuclear RFLP loci. Genetics 139:375–385.
Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.
Rand, D. M., and L. M. Kann. 1998. Mutation and selection at silent and replacement sites in the evolution of animal mitochondrial DNA. Genetica 102/103:393–407.
Raymond, M., and F. Rousset. 1995. GENEPOP (vers. 1.2): population genetic software for exact tests and ecumenicism. J. Hered. 86:248–249.
Reig, O. A. 1989. Karyotypic repatterning as one triggering factor in cases of explosive speciation. Pp. 246–289 *in* A. Fontdevila, ed. Evolutionary biology of transient unstable populations. Springer-Verlag, Berlin.
Reig, O. A., C. Bush, M. O. Ortells, and J. R. Contreras. 1990. An overview of evolution, systematics, population biology cytogenetics, molecular biology and speciation in *Ctenomys*. Pp. 71–96 *in* E. Nevo and O. A. Reig, eds. Evolution of subterranean mammals at the organismal and molecular levels. Wiley-Liss, New York.
Rogers, A. R., and H. Harpending. 1992. Population growth make waves in the distribution of pairwise genetic differences. Mol. Biol. Evol. 9:552–569.
Rousset, F. 1996. Equilibrium values of measures of population subdivision for stepwise mutation processes. Genetics 142: 1357–1362.
Rousset, F., and M. Raymond. 1995. Testing heterozygote excess and deficiency. Genetics 140:1413–1419.
Rozas, J., and R. Rozas. 1999. DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 15:174–175.
Sanguinetti, C. J., E. D. Neto, and A. J. G. Simpson. 1994. Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. Biotechniques 17:915–918.
Schluter, D. 1998. Ecological causes of speciation. Pp. 114–129 *in* D. J. Howard and S. H. Berlocher, eds. Endless forms: species and speciation. Oxford Univ. Press, Oxford, U.K.
Schmidt, P. S., and D. M. Rand. 2001. Adaptative maintenance of genetic polymorphism in an intertidal barnacle: habitat- and life-stage-specific survivorship of Mpi genotypes. Evolution 55: 1336–1344.
Schneider, S., and L. Excoffier. 1999. Estimations of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. Genetics 152:1079–1089.
Schneider, S., D. Roessli, and L. Excoffier. 2000. Arlequin (ver. 2.000): a software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.
Slamovits, C. H., J. A. Cook, E. P. Lessa, and M. S. Rossi. 2001. Recurrent amplifications and deletions of satellite DNA accompanied chromosomal diversification in South American tuco-tucos (genus *Ctenomys*, Rodentia: Octodontidae): a phylogenetic approach. Mol. Biol. Evol. 18:1708–1719.
Slatkin, M. 1985. Rare alleles as indicators of gene flow. Evolution 39:53–65.
———. 1987. Gene flow and the geographic structure of natural populations. Science 236:787–792.

———. 1993. Isolation by distance in equilibrium and nonequilibrium populations. Evolution 47:264–279.

Slatkin, M., and N. H. Barton. 1989. A comparison of three indirect methods for estimating average levels of gene flow. Evolution 43:1349–1368.

Slatkin, M., and R. R. Hudson. 1991. Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. Genetics 129:555–562.

Smith, M. F., and J. L. Patton. 1993. The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the akodontine tribe. Biol. J. Linn. Soc. 50: 149–177.

Sprechmann, P. 1978. The paleoecology and paleogeography of the Uruguayan coastal area during the neogene and quaternary. Zitteliana 4:3–72.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595.

Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22: 4673–4680.

Wakeley, J., and J. Hey. 1997. Estimating ancestral population parameters. Genetics 145:847–855.

Whittam, T. S., A. G. Clark, M. Stonekin, R. L. Cann, and A. C. Wilson. 1986. Allelic variation in human mitochondrial genes based on patterns of restriction site polymorphism. Proc. Natl. Acad. Sci. USA 83:9611–9615.

Wright, S. 1951. The genetical structure of populations. Ann. Eugenics 15:323–354.

Corresponding Editor: T. B. Smith

APPENDIX
Microsatellite allele frequencies across loci and populations.

	Population								
Locus/alleles	Chaparei	Abrojal	Guarida	Tabaré	Las Cañas	Arrayanes	Mafalda	Nuevo Berlín	Total
Hai 2									
1	0.000	0.000	0.000	0.230	0.000	0.000	0.000	0.000	0.029
2	0.000	0.000	0.000	0.000	0.000	0.000	0.300	0.000	0.038
3	0.433	0.000	0.133	0.000	0.000	0.033	0.000	0.000	0.075
4	0.333	0.000	0.000	0.068	0.633	0.000	0.000	0.000	0.129
5	0.200	1.000	0.867	0.000	0.233	0.500	0.667	1.000	0.558
6	0.000	0.000	0.000	0.297	0.133	0.467	0.000	0.000	0.112
7	0.033	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.008
8	0.000	0.000	0.000	0.405	0.000	0.000	0.000	0.000	0.051
Hai 3									
1	0.000	0.000	0.000	0.000	0.000	0.100	0.000	0.000	0.013
2	0.233	0.000	0.000	0.446	0.567	0.033	0.167	0.000	0.181
3	0.267	0.000	0.200	0.554	0.000	0.000	0.133	0.000	0.144
4	0.133	0.400	0.000	0.000	0.000	0.067	0.033	0.000	0.079
5	0.067	0.000	0.000	0.000	0.433	0.800	0.600	1.000	0.363
6	0.167	0.033	0.800	0.000	0.000	0.000	0.067	0.000	0.133
7	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.004
8	0.000	0.533	0.000	0.000	0.000	0.000	0.000	0.000	0.067
9	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004
10	0.100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013
Hai 7									
1	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.004
2	0.200	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.027
3	0.067	0.100	0.967	0.000	0.000	0.000	0.700	0.000	0.229
4	0.133	0.333	0.000	0.824	0.633	0.400	0.167	1.000	0.436
5	0.167	0.533	0.000	0.162	0.000	0.500	0.000	0.000	0.170
6	0.300	0.033	0.000	0.000	0.000	0.100	0.133	0.000	0.071
7	0.100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013
8	0.033	0.000	0.000	0.000	0.367	0.000	0.000	0.000	0.050
Hai 9									
1	0.000	0.400	0.000	0.000	0.000	0.000	0.000	0.000	0.050
2	0.400	0.400	0.400	0.162	1.000	0.333	0.200	1.000	0.487
3	0.500	0.000	0.600	0.000	0.000	0.000	0.600	0.000	0.213
4	0.100	0.200	0.000	0.081	0.000	0.667	0.200	0.000	0.156
5	0.000	0.000	0.000	0.716	0.000	0.000	0.000	0.000	0.090
6	0.000	0.000	0.000	0.041	0.000	0.000	0.000	0.000	0.005
Hai 12									
1	0.000	0.000	0.000	0.000	0.000	0.133	0.000	0.000	0.017
2	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.000	0.004
3	0.300	1.000	1.000	1.000	1.000	0.467	0.667	1.000	0.804
4	0.700	0.000	0.000	0.000	0.000	0.233	0.100	0.000	0.129
5	0.000	0.000	0.000	0.000	0.000	0.133	0.067	0.000	0.025
6	0.000	0.000	0.000	0.000	0.000	0.000	0.167	0.000	0.021
Soc 1									
1	0.267	0.467	0.000	0.000	0.000	0.067	0.167	0.000	0.121
2	0.000	0.000	0.000	0.095	0.000	0.267	0.000	0.000	0.045
3	0.233	0.233	0.000	0.000	0.600	0.167	0.600	1.000	0.354
4	0.100	0.167	1.000	0.905	0.000	0.267	0.000	0.000	0.305
5	0.000	0.000	0.000	0.000	0.400	0.000	0.233	0.000	0.079
6	0.400	0.133	0.000	0.000	0.000	0.000	0.000	0.000	0.067
7	0.000	0.000	0.000	0.000	0.000	0.167	0.000	0.000	0.021
8	0.000	0.000	0.000	0.000	0.000	0.067	0.000	0.000	0.008
Soci 5/6									
1	0.200	0.000	0.000	0.000	0.000	0.036	0.000	0.000	0.030
2	0.000	0.000	0.000	0.527	0.000	0.143	0.133	0.000	0.100
3	0.000	0.000	0.000	0.257	0.000	0.357	0.133	1.000	0.218
4	0.333	0.667	0.800	0.176	0.833	0.179	0.567	0.000	0.444
5	0.133	0.333	0.200	0.000	0.000	0.071	0.100	0.000	0.105
6	0.333	0.000	0.000	0.000	0.167	0.214	0.067	0.000	0.098
7	0.000	0.000	0.000	0.041	0.000	0.000	0.000	0.000	0.005
Hai 6									
1	0.000	0.000	0.000	0.000	0.000	0.067	0.000	0.000	0.008
2	0.000	0.000	0.000	0.333	0.000	0.000	0.100	0.000	0.054
3	0.000	0.000	0.000	0.111	0.083	0.633	0.067	0.000	0.112
4	0.724	0.607	0.179	0.431	0.000	0.067	0.633	1.000	0.455

APPENDIX. Continued.

	Population								
Locus/alleles	Chaparei	Abrojal	Guarida	Tabaré	Las Cañas	Arrayanes	Mafalda	Nuevo Berlín	Total
5	0.172	0.107	0.821	0.000	0.667	0.067	0.100	0.000	0.242
6	0.103	0.250	0.000	0.125	0.000	0.000	0.067	0.000	0.068
7	0.000	0.036	0.000	0.000	0.000	0.167	0.033	0.000	0.030
8	0.000	0.000	0.000	0.000	0.042	0.000	0.000	0.000	0.005
9	0.000	0.000	0.000	0.000	0.208	0.000	0.000	0.000	0.026
Hai 4									
1	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.004
2	0.000	0.000	0.067	0.000	0.000	0.267	0.900	0.000	0.154
3	0.000	0.167	0.000	0.000	0.000	0.033	0.033	0.000	0.029
4	0.000	0.000	0.000	0.297	0.033	0.100	0.000	0.000	0.054
5	0.267	0.133	0.000	0.000	0.000	0.100	0.000	0.000	0.063
6	0.000	0.300	0.000	0.122	0.000	0.000	0.000	1.000	0.178
7	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.000	0.004
8	0.000	0.067	0.000	0.000	0.000	0.000	0.000	0.000	0.008
9	0.000	0.000	0.833	0.135	0.000	0.067	0.000	0.000	0.129
10	0.000	0.067	0.000	0.000	0.267	0.133	0.033	0.000	0.063
11	0.600	0.067	0.000	0.365	0.533	0.100	0.000	0.000	0.208
12	0.133	0.200	0.000	0.081	0.000	0.167	0.033	0.000	0.077
13	0.000	0.000	0.000	0.000	0.167	0.000	0.000	0.000	0.021
14	0.000	0.000	0.067	0.000	0.000	0.000	0.000	0.000	0.008
Soc 2									
1	0.000	0.067	0.000	0.000	0.400	0.033	0.033	0.000	0.067
2	0.600	0.000	0.267	0.162	0.600	0.200	0.233	0.000	0.258
3	0.133	0.900	0.633	0.662	0.000	0.400	0.100	1.000	0.479
4	0.100	0.033	0.100	0.149	0.000	0.133	0.200	0.000	0.089
5	0.067	0.000	0.000	0.027	0.000	0.000	0.433	0.000	0.066
6	0.100	0.000	0.000	0.000	0.000	0.233	0.000	0.000	0.042
Soc 6									
1	0.367	0.000	0.100	0.000	0.300	0.000	0.000	0.000	0.096
2	0.000	0.400	0.000	0.000	0.000	0.000	0.000	0.000	0.050
3	0.633	0.600	0.900	0.432	0.700	0.667	0.633	0.000	0.571
4	0.000	0.000	0.000	0.000	0.000	0.167	0.000	0.000	0.021
5	0.000	0.000	0.000	0.054	0.000	0.000	0.000	0.000	0.007
6	0.000	0.000	0.000	0.284	0.000	0.000	0.000	0.000	0.036
7	0.000	0.000	0.000	0.230	0.000	0.033	0.000	0.000	0.033
8	0.000	0.000	0.000	0.000	0.000	0.000	0.367	1.000	0.171
9	0.000	0.000	0.000	0.000	0.000	0.133	0.000	0.000	0.017