Microsatellite DNA variation in *Spalacopus cyanus*, a social subterranean rodent endemic to Chile (Rodentia: Octodontidae)

Sabine BEGALL[1] & Rodney L. HONEYCUTT[2,3]

[1] University of Essen, Department of General Zoology, Universitätsstraße 5, D–45117 Essen, Germany
[2] Pepperdine University, Natural Science Division, Malibu, CA, USA
[3] corresponding author: rodney.honeycutt@pepperdine.edu

Abstract. We used five polymorphic microsatellite loci to examine patterns of genetic variation within and among colonies of coruros (*Spalacopus cyanus*) at three localities in central Chile. Detailed comparisons of genetic variation at two locations, Los Maitenes in the northern portion of the species' range and El Alamo in the south, displayed very different patterns of variation. Overall variation (alleles per locus and heterozygosity) within and among colonies at Los Maitenes was several times higher than observed at El Alamo, with the latter showing patterns of variation more consistent with a social species of subterranean rodent. Comparisons among Los Maitenes, El Alamo, and Los Vilos, another northern population, revealed a distinct genetic subdivision between northern and southern localities, indicating little evidence of genetic exchange between these two regions. We discuss these patterns of variation in relation to previous genetic research on *Spalacopus* and other subterranean rodents.

Key words. *Spalacopus cyanus*, coruro, population genetics, microsatellites, subterranean rodent, sociality.

INTRODUCTION

The coruro (*Spalacopus cyanus*) is a subterranean rodent endemic to central Chile. Adults range in size from 80–120 g, with short bodies covered in dark brown to black fur (sometimes with blond patches on the belly), sharp claws on the forefeet, short tails, and protruding incisors (Fig. 1; TORRES-MURA & CONTRERAS 1998, VERZI et al. 2015). Unlike some fully subterranean rodents, coruros have well-developed eyes with normal visual acuity (PEICHL et al. 2005). Coruros display a patchy distribution in coastal regions and at lower elevations in the Andes (TORRES-MURA & CONTRERAS 1998, LACEY & EBENSPERGER 2007). Throughout this region, they occupy habitats ranging from grasslands to chaparral to sandy coastal areas (CONTRERAS et al. 1987, TORRES-MURA & CONTRERAS 1998). Despite this habitat diversity and broad distribution, *Spalacopus* shows very little morphological diversity throughout its range. Although there is some evidence for size differences between coastal and Andean populations (REIG et al. 1972, CONTRERAS et al. 1987), comparisons of jaw morphology failed to reveal differences other than variation in incisor resistance among populations occupying soils of different hardness (BACIGALUPE et al. 2002).

Coruros are colonial, with adults, subadults, juveniles, and newborns living in a communal burrow system, with up to 15–26 individuals occupying the same system in some populations

Fig. 1. *Spalacopus cyanus*. Photo by Sabine BEGALL.

(REIG 1970, BEGALL et al. 1999). Colonies display no overlap and occupy a specific home range (BEGALL & GALLARDO 2000). Although coruros housed in the laboratory appear to be more active at night (BEGALL et al. 2002), a radiotelemetry study of free-living animals indicated that activity is restricted to daytime (URREJOLA et al. 2005). As strict herbivores, coruros forage underground for various bulbs and tubers and above ground for leaves, grasses, and forbs, with geophytes representing their staple food (CONTRERAS & GUTIERREZ 1991, BEGALL & GALLARDO 2000). Reproduction occurs throughout the year, with a gestation period of 77 days (BEGALL et al. 1999).

Within the superfamily Octodontoidea, *Spalacopus* (family Octodontidae) and *Ctenomys* (family Ctenomyidae) have independently evolved a truly subterranean lifestyle (HONEYCUTT et al. 2003). Although these two families are closely related phylogenetically, they show markedly different patterns of diversification. *Spalacopus* is a monotypic genus with three subspecies (TORRES-MURA & CONTRERAS 1998) that shows no chromosomal variation throughout its range, with a 2N=58, FN=112 (REIG et al. 1972, GALLARDO 1992). Only two studies of the population genetics and phylogeography of *Spalacopus* have been conducted. GALLARDO et al. (1992) used allozymes to examine patterns of genetic variation in four populations of *Spalacopus* in coastal habitats of central Chile. The allozyme data provided evidence for some genetic subdivisions between northern and southern populations, with the latter showing lower polymorphism and heterozygosity. More recently, OPAZO et al. (2008) used sequence data from a 611 bp portion of the mitochondrial control region to investigate phylogeographic structure in *Spalacopus*. These data revealed overall low levels of nucleotide diversity and the partitioning of variation among three haplotypic groups, northern coastal, central coastal, and southern coastal. F_{ST} (estimate of genetic variation between populations) values were high, with no shared haplotypes among these geographic regions. Thus, both allozyme and mtDNA studies show a pattern of genetic structure expected for subterranean rodents that demonstrate low vagility, a patchy and disjunct distribution, geographic isolation among populations, and the influence of random genetic drift (NEVO 1979, 1995, LESSA 2000).

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In contrast, *Ctenomys* is taxonomically diverse with 69 recognized species (BURGIN et al. 2018) that vary in diploid number from 10 to 70 (REIG et al. 1990, GIMÉNEZ et al. 2002). The phylogenetics, phylogeography, and population genetics of *Ctenomys* have been studied extensively (LESSA & COOK 1998, MORA et al. 2010, PARADA et al. 2011) and a substantial amount of information exists regarding patterns of microsatellite DNA variation in both solitary and social species of *Ctenomys* and other subterranean rodents (e.g., LACEY 2001, QUIRICI et al. 2010, VISSER et al. 2014, INGRAM et al. 2015, MYNHARDT et al. 2021). These studies reveal patterns of variation that are thought to reflect the effects of sociality, dispersal, and environmental factors that potentially influence the long-term stability of colonies. Although *Spalacopus* meets the criteria of a social species (LACY et al. 2019), no genetic data exist to evaluate the effects of this social structure on patterns of variation within and between colonies in a population.

In this paper, we use a panel of microsatellite loci developed from *Spalacopus* (SCHROEDER et al. 2000) to examine patterns of genetic variation among colonies within the same local population as well as among different coastal populations of *Spalacopus*. The specific questions we address include: (1) Does the social structure associated with colony organization influence inter-colony patterns of genetic variation within a population? (2) On a larger geographic scale, does microsatellite genetic variation coincide with the patterns indicated by previous studies based on allozymes and mitochondrial DNA? (3) Are levels of genetic variation within and between populations of *Spalacopus cyanus* like those observed for other rodent species occupying a subterranean niche?

METHODS AND MATERIALS

Localities and samples

Field studies were conducted at two distinct populations in Central Chile (El Alamo, province Ñuble, 36°11'S, 72°28'W, and Los Maitenes, province Valparaíso, 32°45'S, 71°26'W) from October 1997 to March 1998 (Fig. 2). Both study sites were relatively close to the coast (<30 km) and were separated from each other by 400 km. The habitats at the two sites displayed marked differences (Fig. 3A, B; BEGALL & GALLARDO 2000). El Alamo consisted of primarily open pastureland and soils characterized by sand, silt, and a high percentage of clay, supporting numerous geophytes, which are a favorite food of coruros. In contrast, Los Maitenes was a semi-arid area heavily polluted by industrial emissions from a copper processing company. Consequently, the vegetative cover was sparse (<15%), and bulbs that coruros usually consume were absent.

We trapped 138 coruros (El Alamo population – 64; Los Maitenes population – 74). In addition, we examined five animals collected by Milton H. GALLARDO in 1990 from a population in Los Vilos (province Choapa, 31°55'W, 71°31'S), which is located 100 km and 500 km from Los Maitenes and El Alamo, respectively. Capture techniques employed included Victor Oneida No. 0 snap traps, wire traps, and capture by hand. We sacrificed animals used in
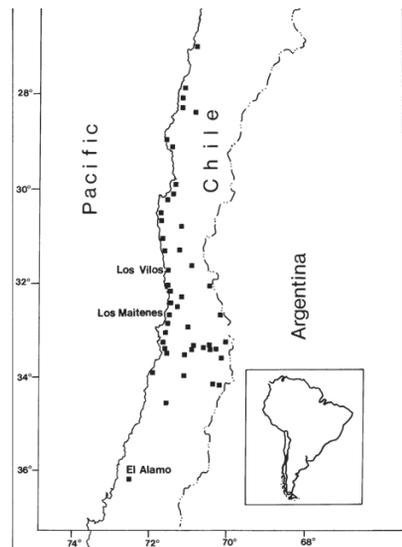

Fig. 2. Distribution of known localities of occurrence of the coruro in Chile. Populations sampled in this study are denoted.
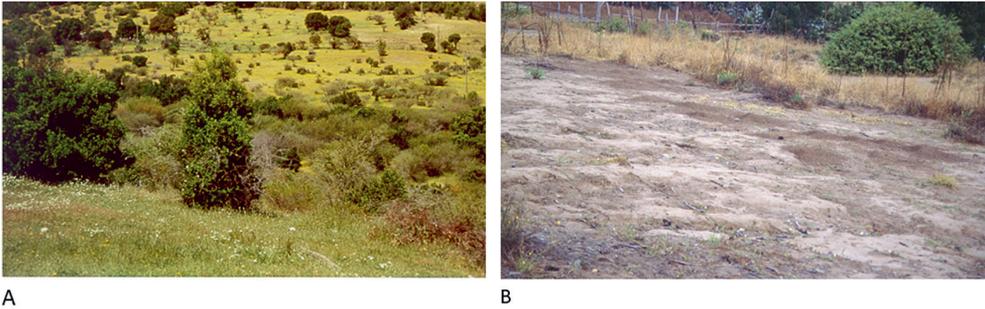
Fig. 3. Habitats at two collecting sites for *Spalacopus cyanus*; A – El Alamo site in southern central Chile, B – Los Maitenes site in northern Chile. Photos by Sabine BEGALL.

our genetic research with a ketamine overdose and stored liver and skin samples in 95% ethanol. Animal handling protocols followed those outlined in SIKES et al. (2016).

To avoid potential demographic biases when comparing populations, we excluded samples of embryos/fetuses, newborns, and juveniles from our genetic analyses. Consequently, individuals used in genetic comparisons consisted of 33 individuals from El Alamo, 55 from Los Maitenes, and 5 from Los Vilos. We examined intra- and inter-population variation using comparisons of five colonies (Ant1, Ant2, Ant8, Jose, Juan) from the El Alamo population and six colonies (LM1, LM2, LM3, LM4, LM11, LM12) from the Los Maitenes population. Colonies at both the El Alamo and Los Maitenes populations showed no overlap with neighboring burrow systems, a distinct system of mounds, and displayed clear spatial separation (BEGALL & GALLARDO 2000). We used individuals from one colony at Los Vilos population for broader geographic comparisons.

Geographic distances between colonies as determined with a GPS navigation system (Garmin) were, on average, 247±168 m at El Alamo (range: 30–530 m, N=5) and 313±168 m at Los Maitenes (range: 60–620 m, N=6).

Extraction of DNA and analysis

After grinding tissue samples in liquid nitrogen, we used a phenol-chloroform-isopropanol extraction to isolate genomic DNA (SAMBROCK et al. 1989). Individuals were genotyped using six microsatellite loci developed specifically for *Spalacopus cyanus*; PCR amplification conditions and genotyping protocols are the same as those in SCHROEDER et al. (2000).

Statistics

We examined patterns of genetic variation at two spatial scales – inter-colony variation within the El Alamo and Los Maitenes populations and broader inter-population comparisons involving El Alamo, Los Maitenes, and Los Vilos.

We used GenAlEx 6.502 (PEAKALL & SMOUSE 2012) to estimate observed and expected heterozygosity, and number of alleles per locus. We tested for departures from Hardy-Weinberg equilibrium in GenAlEx and used an excel calculator (GAETANO 2018) to adjust for multiple tests following Holm's sequential Bonferroni method (HOLM 1979). Other analyses performed in GenAlex included an Analysis of Molecular Variance (AMOVA), a Principal Coordinates Analysis (PCoA), and Population Assignments. The AMOVA provides information on partitioning of genetic variation within and among populations (EXCOFFIER

Table 1. Microsatellite variation in *Spalacopus cyanus*; N = number of individuals; Na = number of alleles per locus; Ho = observed heterozygosity; He = expected heterozygosity

population	N	Na	Ho	He
El Alamo	33	1.6	0.367	0.235
Ant8	9	1.4	0.222	0.158
Juan	8	1.6	0.425	0.286
Jose	11	2.0	0.588	0.389
Ant1	3	1.4	0.200	0.144
Ant2	2	1.4	0.400	0.200
Los Maitenes	55	3.4	0.716	0.567
LM1	10	3.2	0.618	0.497
LM3	4	3.2	0.800	0.625
LM4	4	3.2	0.750	0.556
LM11	28	4.8	0.592	0.604
LM12	6	3.0	0.667	0.508
LM2	3	3.0	0.867	0.611

et al. 1992); for this analysis, we compared a distance matrix based on F_{ST} values to estimate how much variation resulted from differences among populations. Genetic distances were calculated (Nei 1978), after which the PCoA was used to visualize genetic distances among individuals from different colonies or populations. The population assignment method uses allele frequency data and individual genotypes to assign individuals to the most likely population of origin. We used GENEPOP (Raymond & Rousset 1995, Rousset 2008) to estimate the number of migrants (Nm) based on the methods described in Barton & Slatkin (1986) and Slatkin (1995). We used PAUP* (Swofford 2002) to construct a phenogram based on Nei's distance (Nei 1978) and neighbor-joining. Finally, we tested for isolation by distance using the Mantel test (Mantel 1967) in GenAlex 6.502, which compares matrices of F_{ST} and geographic distance between colonies in both the El Alamo and Los Maitenes populations. All means are given ±1 SD.

RESULTS

Inter-colony comparisons per population

One locus (SB111) was monomorphic for the populations examined. The remaining five loci were polymorphic within the El Alamo and Los Maitenes populations; none of these loci displayed departures from Hardy-Weinberg equilibrium in either population. Inter-colony comparisons for the El Alamo and Los Maitenes populations showed different patterns of genetic variation (Table 1, Fig. 4). Based on a Mann-Whitney U test, the Los Maitenes population had a significantly higher number of alleles per loci (U=0, $p<0.01$) and significantly greater observed heterozygosity (U=0, $p<0.01$) relative to the El Alamo population. In contrast, 91% of individuals in the El Alamo population were correctly assigned to the colony from which they were captured, and only 44% of individuals at Los Maitenes were assigned to their colony of origin. Among-colony differences accounted for 28% of the total genetic variability detected at El Alamo (AMOVA, $p<0.0001$) and 6% of the total variability at Los Maitenes (AMOVA, $p<0.0001$).

Estimates of among-colony F_{ST} values and geographic distances among colonies in each population revealed a different pattern (Table 2, Fig. 4). El Alamo had significantly higher F_{ST}
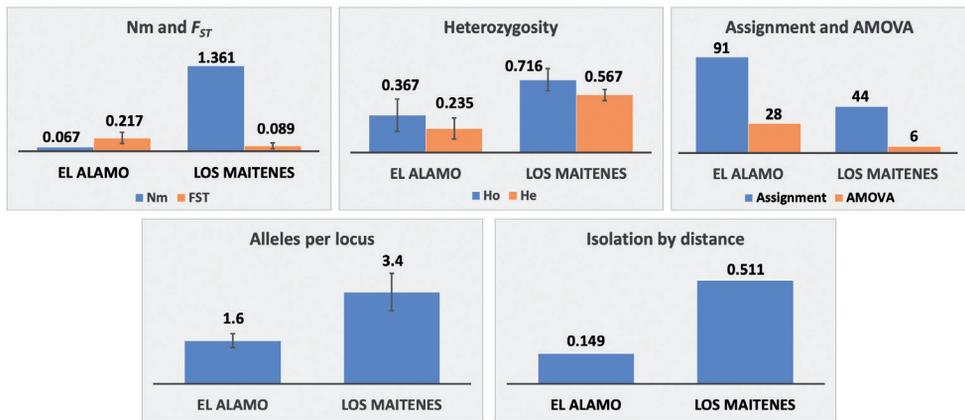
Fig. 4. Comparison of inter-colony variation in El Alamo and Los Maitenes populations; the numbers used for isolation by distance represent R^2 values. Error bars indicate standard deviations.

values relative to Los Maitenes (U=14.5, $p<0.01$), although the mean distance between colonies in the El Alamo (247±168 m) and Los Maitenes (313±168 m) populations were not significantly different (U=57.5, $p=0.347$). Estimates of the number of migrants per generation (Nm – after correction for size) among colonies in the El Alamo population was 0.0669, and Nm was 1.3609 in the Los Maitenes population. The Mantel test indicated no significant isolation by distance within the El Alamo population ($R^2=0.1499$, $p=0.211$) but significant isolation by distance at Los Maitenes ($R^2=0.511$, $p=0.022$).

Table 2. Inter-colony comparisons based on estimates of F_{ST} (above diagonal) and geographic distances (meters) between colonies (below the diagonal)

El Alamo	Ant8	Juan	Jose	Ant1	Ant2	
Ant8	–	0.103	0.267	0.231	0.162	
Juan	170	–	0.181	0.211	0.084	
Jose	440	270	–	0.299	0.263	
Ant1	90	260	530	–	0.365	
Ant2	30	150	420	110	–	
Los Maitenes	LM1	LM2	LM3	LM4	LM11	LM12
LM1	–	0.127	0.152	0.054	0.045	0.068
LM2	260	–	0.148	0.102	0.08	0.103
LM3	410	430	–	0.119	0.122	0.140
LM4	300	220	620	–	0.029	0.014
LM11	60	280	470	270	–	0.028
LM12	230	210	578	90	203	–

Table 3. Comparison of microsatellite variation in *Spalacopus* to other species of solitary and social subterranean rodents; Na = number of alleles per locus; Ho = observed heterozygosity; He = expected heterozygosity; asterisk (*) denotes species that maintain colonies containing individuals that cooperate in tunnel construction and foraging for food and remain together for multiple generations

| species	#Loci	Na	Ho	He	social structure	reference
Ctenomys australis	8	4.8	0.587	0.522	solitary	Mora et al. (2010)
Ctenomys haigi	15	6.8	0.688	0.706	solitary	Lacy (2001)
Ctenomys magellanicus	5	8.6	0.522	0.638	solitary	Fasanella et al. (2013)
Ctenomys minutus	6	9.3	0.495		solitary	Gava & Freitas (2004)
Ctenomys sociabilis	15	2	0.078	0.078	social*	Lacy (2001)
Ctenomys talarum	12	4.9	0.480	0.515	solitary	Cutrera et al. (2006)
Spalacopus cyanus	5	2.6	0.557	0.416	social*	this study
Octodon degus	6	6.6	0.538	0.745	social	Quirici et al. (2010)
Heterocephalus glaber	12	4.6	0.262	0.297	social*	Ingram et al. (2015)
Bathyergus suillus	9	17.8	0.599	0.858	solitary	Visser et al. (2014)
Fukomys damarensis	7	13.6	0.781	0.842	social*	Mynhardt et al. (2021)
Geomys breviceps	14	4.6	0.611	0.610	solitary	Welborn & Light (2014)
Spalax ehrenbergi	19	7.1		0.812	solitary	Karanth et al. (2004)
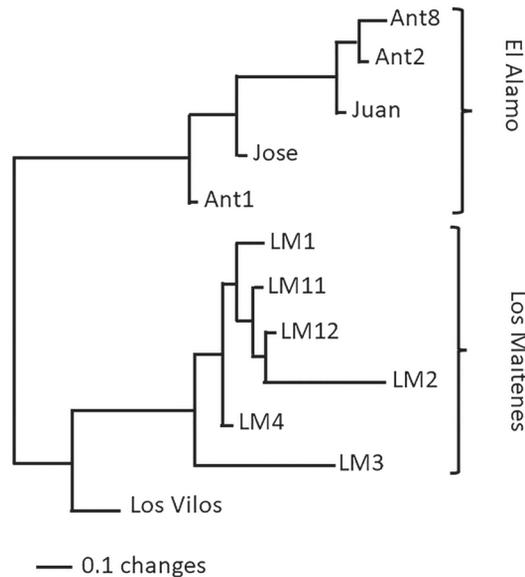
Fig. 5. Phenogram based on genetic distances according to Nei (1978) and a neighbor-joining analysis in PAUP* showing relationships among all colonies examined.

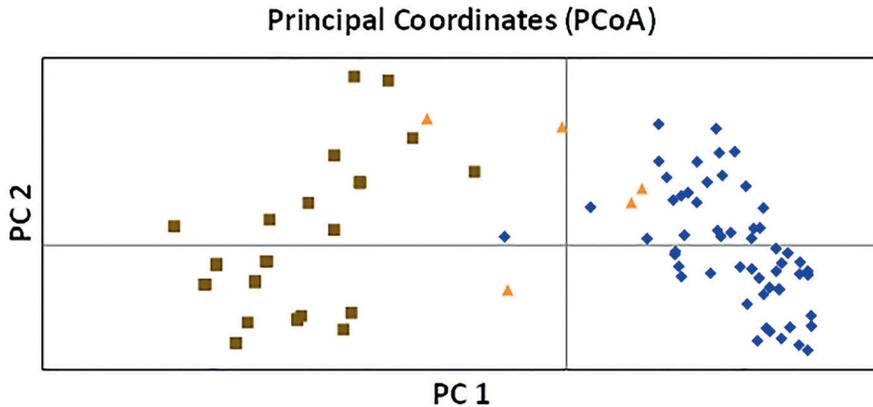## Principal Coordinates (PCoA)

Fig. 6. Principal Coordinate Analyses of genetic distances for Los Maitenes (blue diamonds), Los Vilos (orange triangles), and El Alamo (brown squares).

Inter-population comparisons

Pairwise comparisons of F_{ST} values revealed that differentiation was greatest between El Alamo and Los Maitenes (F_{ST}=0.271), followed by El Alamo and Los Vilos (F_{ST}=0.176), with the least differentiation between Los Maitenes and Los Vilos (F_{ST}=0.094). Los Maitenes contained a total of nine private alleles versus two at El Alamo and five at Los Vilos. The estimated number of migrants (Nm – after correction for size) between El Alamo and Los Maitenes was 0.1295, suggesting low levels of gene flow.

Overall, 97% of individuals at El Alamo, Los Maitenes, and Los Vilos populations were correctly assigned to their population of origin. Nei's D between Los Maitenes and El Alamo was 1.233; values for D between these populations and Los Vilos were 0.533 and 0.643, respectively. The neighbor-joining analysis of Nei's D revealed a major separation between Los Maitenes and El Alamo, with the Los Vilos population grouping closer to Los Maitenes (Fig. 5). The AMOVA results indicated that 35 % of the total genetic variation detected occurred among populations. The Principal Coordinates Analysis (PCoA) based on genetic distance revealed a separation between the El Alamo and Los Maitenes populations, with individuals from Los Vilos being intermediate (Fig. 6). Between colony comparisons for Los Maitenes showed a tighter clustering than seen for El Alamo.

DISCUSSION

Inter-colony genetic differences

According to LACEY et al. (2019), *Spalacopus cyanus* fits the requirements for a social species. Coruros share a communal burrow (both night and day), and individuals in the colony consist of adults, subadults, and recent offspring. Individual burrow systems or colonies do not overlap with one another (BEGALL & GALLARDO 2000), and members of a colony demonstrate aggressiveness toward individuals from other colonies (TORRES-MURA & CONTRERAS 1998). These

behavioral patterns suggest some degree of intra-colony fidelity and low dispersal between colonies. Therefore, patterns of genetic variation should reflect colony fidelity.

Surprisingly, inter-colony comparisons of genetic variation at the El Alamo and Los Maitenes populations show markedly different patterns (Table 1, Figs. 4, 6). El Alamo colonies were characterized by lower average Nm values, suggesting low levels of gene flow between colonies, resulting in higher levels of inter-colony differentiation, as reflected in the AMOVA and the PCoA results. In contrast, inter-colony genetic variation at Los Maitenes was less pronounced, suggesting more inter-colony dispersal and reduced colony fidelity. Based on these comparisons, the El Alamo population displays a pattern of inter-colony genetic variation that might be expected for a social species, whereas Los Maitenes displays a pattern suggesting considerably less genetic separation between colonies.

What explains the differences in inter-colony variation seen in these two populations? Reig (1970) proposed that coruros are nomadic, with colonies moving in response to the depletion of resources. Therefore, environmental differences between habitats and access to resources at El Alamo and Los Maitenes may provide one explanation. As noted by Begall & Gallardo (2000), colonies at these two sites show differences in food availability and plant diversity. Los Maitenes is highly polluted and devoid of geophytes. Individuals in this population are known to forage above ground on leaves and other plant matter more than the animals at El Alamo. These differences in environmental quality or habitat type may lead to more aboveground foraging, greater dispersal, and decreased colony stability at Los Maitenes, thereby potentially contributing to the contrasting patterns of genetic variation reported here. Other studies of octodontid rodents have noted the potential effect of habitat. *Octodon degus* forms social groups, but turnover of these groups is high and may be related to the habitat (Ebensperger et al. 2009). Mora et al. (2010) also noted the potential role that habitat configuration plays in influencing patterns of genetic variation in *Ctenomys australis*.

Few studies have characterized intra-specific differences in ecology between coruro populations occupying different habitats. However, the study by Lacey et al. (2019) provides some intriguing information. Their study showed differences in home range size and degree of overlap among groups occupying distinct clusters in contrastingly different habitat types. Given the broad range of habitat types occupied by coruros, populations may show marked intra-specific differences in patterns of colony structure and genetic variation.

Patterns of divergence between populations

The patterns of microsatellite variation reported here are consistent with previous studies of allozymes (Gallardo et al. 1992) and mitochondrial DNA (mtDNA) sequences (Opazo et al. 2018). Gallardo et al. (1992) indicated higher levels of intra-colony diversity in three northern populations (Los Vilos, Los Cristales, and Huentelauquén) versus our southern population (Quirihue = El Alamo in our study). More specifically, the northern population contained three times more polymorphic loci (northern = 26.3%, southern = 8.7%) and twelve times more heterozygosity (northern = 7.3%, southern = 0.6%) than the Quirihue population. Opazo et al. (2008) reported that the Quirihue (= El Alamo) population contained only one mitochondrial haplotype representing one of the three haplotype groups, with northern clades representing a second haplotype group.

Based on known populations of *Spalacopus*, there appears to be a 200 km gap between El Alamo and the closest northern population (Santa Cruz), with no intervening colonies known

(Fig. 2; Verzi et al. 2015). The naked mole-rat, *Heterocephalus glaber*, displays a somewhat similar pattern of genetic variation. In the southern portion of their range, populations of mole-rats show low levels of microsatellite and mtDNA variation, possibly in response to a potential founder event or population bottleneck (Ingram et al. 2015). Similar historical conditions, resulting in a population bottleneck or founder event may also have contributed to the reduced variation in the southern-most population of *Spalacopus* examined, thus potentially explaining the difference between southern and northern populations.

Comparison to other subterranean rodents

How do patterns of genetic variation in *Spalacopus* compare to other subterranean rodents? *Spalacopus* tends to have low numbers of alleles per locus and low levels of heterozygosity, like that observed for solitary and social species of subterranean rodents (Table 3). The geographic variation in *Spalacopus* tends to reflect variation expected for a species displaying low vagility and habitat fragmentation (Lessa 2000). If we examine the El Alamo and Los Maitenes populations separately (Table 1, Fig. 4), El Alamo shows some of the lowest levels of genetic variation, except for *Ctenomys sociablis* (a social species of ctenomyid) and *Heteroceophalus glaber* (considered eusocial). Therefore, colony fidelity, resulting in less inter-colony dispersal, appears to have influenced patterns of variation at Los Alamos.

CONCLUSIONS

All genetic data collected to date point to a difference between the southern and northern populations of *Spalacopus cyanus*. Hopefully, more extensive collecting in the future and the inclusion of more genetic and ecological information will help provide a more thorough assessment of geographic variation in this species. In addition, the contrasting patterns of variation between colonies occupying different habitats suggest the potential role of ecological factors on the long-term stability of social groups. We suggest that studies of social behavior and genetic variation in divergent habitat types may provide a more detailed explanation for the patterns seen for *Spalacopus*.

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