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Artículo

GENETIC ANALYSES SUGGEST BURROW SHARING BY RÍO NEGRO TUCO-TUCOS (*Ctenomys rionegrensis*)

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ABSTRACT. Genetic analyses of kinship can generate important insights into social structure, particularly for species for which direct observations of social relationships are challenging. We used molecular markers to characterize the kin structure of a population of the Río Negro tuco-tuco (*Ctenomys rionegrensis*), a subterranean species of rodent that is rarely observed above ground. Previous research has revealed that adults of this species engage in at least periodic episodes of burrow sharing, indicating that *C. rionegrensis* may not be strictly solitary. To explore the kin structure of this species, we used variability at 10 microsatellite loci to determine if (1) adults and juveniles captured at the same burrow entrance were parents and offspring and (2) kinship among adults captured together differed from that among randomly sampled pairs of individuals in our study population. Our analyses revealed that adults and juveniles captured together were not typically parents and offspring, suggesting potential mixing of litters among burrow systems. Relatedness among adults captured together did not differ from background levels of genetic similarity, providing no evidence that spatial proximity was associated with kin structure. Collectively, our findings support the hypothesis that *C. rionegrensis* is not strictly solitary but instead engages in burrow sharing by adults and associated litters of young.

RESUMEN. Análisis genéticos sugieren la existencia de madrigueras compartidas en los tucu-tucus de Río Negro (*Ctenomys rionegrensis*). Los análisis genéticos de parentesco pueden generar importantes ideas sobre la estructura social, particularmente en especies donde las observaciones directas de las relaciones sociales son aún muy discutidas. Utilizamos marcadores moleculares para caracterizar la estructura de parentesco en una población de los tuco-tucos de Río Negro (*Ctenomys rionegrensis*), una especie de roedores subterráneos que rara vez se observa sobre la superficie. Investigaciones anteriores han revelado que los adultos de esta especie pueden compartir madrigueras, al menos por periodos, indicando que esta especie puede no ser estrictamente solitaria. Para explorar la estructura de parentesco de esta especie utilizamos la variabilidad presente en 10 loci de microsatélites para determinar si (1) adultos y juveniles capturados en la misma madriguera son padres e hijos y (2) el parentesco entre los adultos capturados en las mismas cuevas difiere del de pares de individuos muestreados al azar en la población estudiada. Nuestros análisis revelaron que los adultos y los juveniles capturados juntos típicamente no están emparentados, lo que sugiere una posible mezcla de camadas dentro del sistema de madrigueras. El parentesco entre los adultos capturados juntos, sin embargo, no difirió de los niveles medios de similitud genética, sin proporcionar evidencia de que la proximidad espacial estuviera asociada con la estructura de parentesco. En conjunto, nuestros hallazgos apoyan la hipótesis de que *C. rionegrensis* no es una especie estrictamente solitaria, sino que existe intercambio de madrigueras entre los adultos y sus camadas asociadas.

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Palabras clave: madrigueras compartidas, *Ctenomys rionegrensis*, Uruguay,

INTRODUCTION

Kinship is a fundamental component of many mammalian societies, with critical implications for the fitness consequences of interactions among conspecifics (Armitage 1987; Clutton-brock 2002). In group-living species, kinship among group mates may be associated with specialized forms of social behavior (e.g., cooperative breeding) that do not occur among unrelated individuals (Emlen 1995; Cluttion-brock & D. Lukas 2012). Although adults in solitary species generally live alone, the distinctively mammalian trait of lactation (Pond 1997) suggests that juveniles interact with close kin (e.g., mothers and littermates) and these interactions may influence adult social relationships (Sherman 1981). Thus, kinship plays a central role in the behavior of both social and solitary species and analyses of kinship are an important part of efforts to characterize mammalian social structure.

In subterranean mammals, sociality occurs when multiple adults share the same burrow system (Nevo 1979; Lacey 2001). Among subterranean rodents, sociality has been documented in several lineages, including African mole-rats (Bathyergidae: Honeycutt 1992; Bennett & Faulkes 2002) and tuco-tucos (Ctenomyidae: Lacey et al. 1997; Lacey 2000). In those social subterranean species for which appropriate data are available, burrow mates of one or both sexes tend to be close kin. For example, in naked (*Heterocephalus glaber*) and Damaraland (*Fukomys damarensis*) mole-rats, all adults within a group tend to be closely related, as expected given that both males and females are philopatric (Reeve et al. 1990; Bennett & Faulkes 2002). In contrast, in colonial tuco-tucos (*Ctenomys sociabilis*), only the adult females in a group are kin; while females in this species are philopatric, males are not (Lacey & Wieczorek 2004). Thus, in addition to providing critical information regarding group structure, analyses of genetic kinship among individuals can generate important insights into the demographic patterns giving rise to that structure.

The social structures of many subterranean rodent species remain unknown (Lacey 2000). For others, anecdotal reports suggest burrow sharing by adults, although quantitative evidence of sociality is lacking (Lacey 2000; Lacey & Sherman 2007). One such species is the Río Negro tuco-tuco, *C. rionegrensis* (Reig et al. 1990). These animals are endemic to western Uruguay, where they inhabit relict sand dunes along the shores of the Río Negro and Río Uruguay (Langguth & Abella 1970). Initially, field studies of *C. rionegrensis* revealed that multiple adults could be captured at a single burrow entrance (Lessa et al. 2005). Subsequent radiotelemetry studies (Tassino et al. 2011) identified several apparent examples of overlap among the home ranges of different adults, including adult females as well as opposite-sex adults. Unlike the group-living *C. sociabilis*, however, *C. rionegrensis* is not known to display several conspicuous signals of sociality such as multiple adults foraging together at the same burrow entrance or alarm calling to conspecifics in response to predators (Pearson & Christie 1985; Lacey & Ebensperger 2007). As a result, additional data are needed to understand fully the social structure of the Río Negro tuco-tuco.

To evaluate reports of burrow sharing in this species, we assessed patterns of relatedness among *C. rionegrensis* captured together. Specifically, we used microsatellite markers developed for *Ctenomys* to quantify the degree of genetic kinship among individuals captured at the same burrow entrance. Our findings support previous reports of burrow sharing in *C. rionegrensis* but reveal apparent differences in kin structure between this species and the group-living *C. sociabilis*. Collectively, these data suggest that the social structure of *C. rionegrensis* differs from that of other tuco-tucos studied to date, thereby adding to the growing comparative picture of ctenomyid social behavior.

MATERIALS AND METHODS

Study site

The study population was located on Estancia El Tabaré, Departamento de Río Negro, Uruguay (33°21.41'S, 58°18.57' W; **Fig.** 1). This region consists of a series of old sand dunes located between the Río Negro and the Río Uruguay. Mean annual precipitation at the study site was 1130 mm per year, with a mean monthly temperature of 21.8 °C. The focal study area measured approximately 200 m by 300 m. Vegetation on the study area consisted of a mixture of annual grasses and woody shrubs; in general, vegetative cover was sparse and patchily distributed.

Animal capture and tissue collection

Fieldwork was conducted from October to December 1999. Animals were captured with Sherman-like live traps that

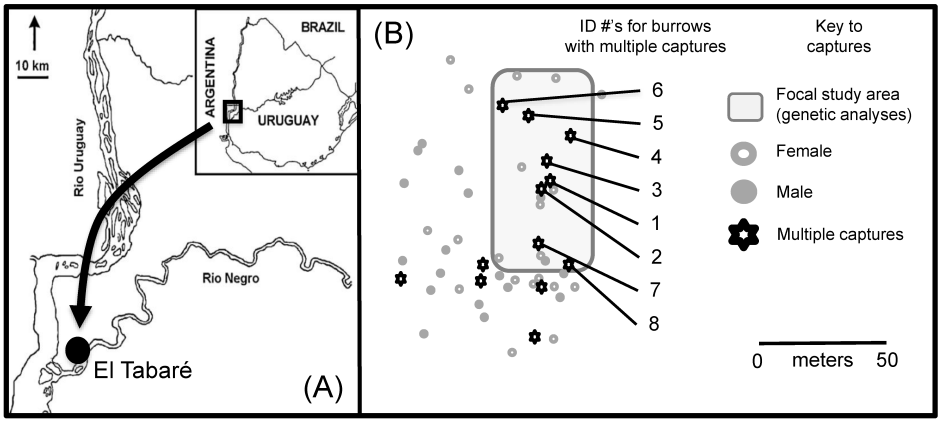

Fig. 1. Map of the study area showing (A) the location of Estancia El Tabaré in Departamento de Río Negro, Uruguay. In (B), the relative locations of the burrow entrances at which *C. rionegrensis* were captured are shown for the entire study site as well as for the portion of the site (gray rectangle) for which genetic analyses were completed. Entrances at which multiple individuals were captured are indicated in bold; the 24 animals caught at the burrow entrances numbered 1 to 8 were used in genetic analyses of kinship.

had been constructed specifically for use with the study species. Traps were set at all burrow entrances characterized by fresh soil plugs or freshly excavated mounds of dirt; each burrow entrance was opened and a trap was inserted into the adjacent tunnel. Traps were checked at least every 2 h and captured animals removed as soon as they were detected. To insure that all individuals in a burrow system were captured, traps were reset at burrow entrances at which animals had been caught. Trapping of a given burrow entrance continued until no activity (additional captures, plugging of the burrow entrance) had been detected at that location for at least 12 h. The location of each capture was recorded to the nearest meter using established landmarks on the study site.

For all animals captured, we recorded sex and body weight (300 ± 2 g Avinet scale). In addition, for females, we assessed reproductive status based on external cues such as the appearance of the vagina and the degree of development of the mammae. No females weighing less than 100 g displayed evidence of reproductive activity and thus individuals below this body weight were considered to be juveniles. Like other ctenomyids, male *C. rionegrensis* do not display external cues (e.g., descended testes) that can be used to distinguish reproductive from non-reproductive males. Instead, we used the criterion established by Tassino & Passos (2010) for *C. rionegrensis*–based in part on data from our study population– that only males weighing more than 120 g were considered adults.

Captured individuals were euthanized and samples of liver tissue were collected for use in studies of the evolutionary genetics of *C. rionegrensis* (Wlasiuk et al. 2003; Lessa et al. 2005). A subset of tissue samples representing 24 individuals captured at 8 burrow entrances were analyzed as part of this study (**Table** 1 and **Fig.** 1). All procedures involving live animals followed the guidelines established by the American Society of Mammalogists for the use of wild mammals in research (Sikes et al. 2016).

DNA extraction and microsatellite typing

Genomic DNA was isolated from liver samples following a slightly modified version of the protocol in Miller et al. (1988). Seven microsatellite loci developed for *C. sociabilis* (Soc1, Soc2, Soc3, Soc7, Soc8; Lacey 2001: Soc5, Soc6; E. Lacey, unpubl. data) and four microsatellite loci developed for *C. haigi* (Hai 3, Hai 4, Hai 9, Hai 11, Hai 12; Lacey et al. 1999) were used to characterize genetic variation in the study animals. These loci were selected for analysis based on a previous study indicating that they are polymorphic in *C. rionegrensis* (Wlasiuk et al. 2003). PCR amplifications were conducted using 8 μl reaction volumes consisting of 0.4 U of Taq Polymerase (Biotools), 0.8 μl of Buffer (10 X, 20 mM MgCl2), 0.16 μl of each primer (10 mM each), 0.16 μl of dNTPs (10 mM each) and 2 μl of DNA template, with one primer per pair fluorescently labeled with HEX, FAM, or TET. Amplifications were conducted in a Thermo Hybaid PXE 0.2 Thermal Cycler using the following conditions: initial denaturation at 94°C for 4 min followed by 34 cycles of denaturation at 94°C for 30 s, annealing temperature for 30 s, and extension at 72°C for 45 s, with a final extension at 72°C for 5 min. Locus-specific annealing temperatures are given in **Table** 2; for Soc7 and Soc8, the first 10 cycles were conducted at Ta = 49°C, with the remaining cycles conducted at Ta = 48°C. PCR products were electrophoresed on an ABI 3130 Genetic Analyzer housed in the Unidad de Biología Molecular of the Institut Pasteur (Montevideo, Uruguay). A LIZ500 (-250) size standard was included in all lanes. Fragment sizes were determined and genotypes were assigned using the Peak Scanner Software v1 software (Applied Biosystems).

Statistical analyses

For each microsatellite locus analyzed, observed allelic and genotypic frequencies were calculated and expected heterozygosity was estimated using GENEPOPV4 (Rousset 2008). The same program was used to identify potential

departures from Hardy-Weinberg (HW) expectations and to test for potential linkage disequilibrium (LD) among loci. For both HW and LD analyses, estimated values were based on 10 000 Markov chain iterations. To account for the repeated used of these tests, a Bonferroni correction (Rice 1989) was applied to the alpha values used to assess the significance of these analyses.

Kinship among individuals was assessed using three different approaches. First, parentage exclusion analyses were conducted by manually comparing the microsatellite genotypes of juveniles and adults captured in the same burrow system; adults that could not have contributed the alleles present in a juvenile were excluded as potential parents of that individual if a mismatch occurred at one or more loci. Second, we analyzed microsatellite genotypes using CERVUS 3.0 (Marshall et al. 1998; Kalinowski et al. 2007), which employs a maximum likelihood approach to determine which of a candidate set of animals are most likely to be the true parents of an individual. Only adults assigned as parents with > 95% confidence were retained for subsequent analyses of kinship.

Finally, for all pair-wise combinations of individuals captured together, we used Kingroup v2 (Konovalov et al. 2004) to calculate coefficients of relatedness (r-values) based on microsatellite genotypes. Although no biologically confirmed parent-offspring pairs (e.g., pregnant female and fetuses) were available for analysis, r-values among parent-offspring or full siblings pairs are expected to approach 0.5; as a result, observed r-values were tested against the expectation of r = 0.50 using the likelihood algorithm contained in Kingroup. To compare r-values generated for animals captured together to the overall level of relatedness in our study population, we also calculated pairwise r-values for 30 randomly generated pairs of individuals not captured at the same burrow entrance.

Comparisons of r-values generated for different categories of individuals were conducted using standard two-sample analyses. In general, data on kinship were not normally distributed and thus non-parametric tests Mann-Whitney U tests were used for most analyses.

RESULTS

A total of 143 animals (41 adult males, 59 adult females, 24 juvenile males, 17 juvenile females, 2 juveniles for which sex was not known) was captured at 114 distinct burrow entrances located throughout the study site. Multiple individuals (22 adults, 24 juveniles) were captured at 17 (14.9%) of these burrow entrances, for a mean of 2.7 $\pm$ 0.8 animals (range = 2-4) per entrance at which multiple captures occurred. At 12 (70.6%) of these burrow entrances, the animals captured consisted of one adult female and one or more juveniles. At the remaining five burrow entrances, the animals captured consisted of juveniles and an adult male (N = 1 entrance), juveniles and multiple adult females (N = 1 entrance), or juveniles and adults of both sexes (N = 3 entrances). Successive captures at the same location typically occurred within a few hours of each other during the same day of trapping, thereby minimizing the likelihood that these findings resulted from immigration from other burrow systems.

Table 1

Summary of captures at 8 burrow entrances at which multiple *C. rionegrensis* were caught. For each individual captured, relative age (A = adult, J = juvenile), sex (F = female, M = male), and body weight in grams are reported, as is the identification number (ID) given to each animal.

Burrow Entrance	Relative age	Sex	Weight (g)	Animal ID
1	A	F	138	1170
	A	M	190	1188
	A	M	158	1209
	J	F	58	1171
2	A	F	134	1172
	J	M	38	1181
	J	F	38	1175
	J	F	42	1173
3	A	F	150	1194
	J	F	58	1197
4	A	M	194	1131
	A	F	164	1148
5	A	F	158	1151
	J	F	66	1152
6	A	M	148	1155
	J	M	82	1165
	J	F	76	1164
7	A	M	196	1193
	A	F	165	1183
	J	M	32	1212
8	A	F	159	1213
	J	M	70	1216
	J	M	93	1215
	J	F	67	1214

Microsatellite variability

Microsatellite genotypes were generated for 24 individuals captured at 8 burrow entrances (**Fig.** 2). Number of alleles, allele frequencies, and observed and expected heterozygosity at each locus are shown in **Table** 2. One locus (Soc5) was monomorphic for the animals analyzed and was excluded from further analyses. After Bonferroni correction (corrected alpha = 0.005), none of the remaining loci displayed significant departures from Hardy-Weinberg expectations (**Table** 2). Similarly, after Bonferroni correction, no evidence of linkage disequilibrium among loci was evident (all p > 0.0008). The polymorphic information content for the final data set

(N = 10 loci after exclusion of Soc5) was 0.355 and the probability of exclusion was 0.99, suggesting that these markers were appropriate for analyses of parentage and kinship (Marshall et al. 1998; Slate et al. 2000).

Parentage analyses

Paternity exclusion analyses based on direct visual comparisons of genotypes indicated that for 4 (25.0%) of the 16 adult-juvenile pairs captured together, the adult could not be the parent of the juvenile with which it was caught. This included 3 adult male-juvenile pairs captured together (**Fig.** 2). Parentage assignment analyses revealed that of the remaining 12 pairs of adults and juveniles captured together, the adult in question could be assigned (> 95% probability) as the parent of that juvenile in only 3 (25.0%) cases. Thus, overall, more than three-quarters (13 of 16, or 81.3%) of the adult-juvenile pairs caught at the same burrow entrance did not appear to consist of parents and offspring. For 3 (27.3%) of the juveniles included in our genetic analyses, the adults identified as the parents of these individuals were captured at a different burrow entrance than the juvenile to which they were assigned. In these three cases, each parent (mother and father) was caught at a different burrow entrance and was captured with other individuals to which they were not assigned as parents. Collectively, these findings reveal that capturing adults and juveniles at the same burrow entrance did not provide a reliable indicator of the genetic parentage of young.

Kinship among individuals captured together

Mean pairwise relatedness between members of 30 randomly selected pairs of animals was 0.250 ± 0.180 (range = 0.004 to 0.790). Among individuals captured at the same burrow entrance, mean relatedness was 0.247 ± 0.183 (N = 27 pairs; range = 0.001 to 0.569; **Fig.** 3); the difference in values for these pairs versus randomly generated pairs was not significant (Mann-Whitney U test, Z = 0.39, two-tailed p = 0.70). When estimates of relatedness for animals captured together were examined as a function of age class, we found that mean relatedness was lowest between pairs of adults (0.087 ± 0.076, N = 6), higher between adults and juveniles (0.275 ± 0.153, N = 16), and highest between pairs of juveniles (0.321 ± 0.184, N = 5; **Fig.** 3). Consistent with this, r-values for 5 (83.3%) of the 6 pairs of adults captured together were significantly less than 0.50 (**Fig.** 2); in contrast, only 7 (43.8%) of 16 r-values for adult-juvenile pairs and 2 (40.0%) of 5 r-values for juvenile-juvenile pairs were significantly less than 0.5 (**Fig.** 3).

When estimates of kinship were examined as a function of parentage, we found that mean relatedness between adults and juveniles captured together was highest for pairs for which the adult had been assigned as the parent of the juvenile in question (0.470 ± 0.150, N = 3). In contrast, mean relatedness was markedly lower for pairs for which the adult was excluded as the parent of the juvenile (0.139 ± 0.131; N = 4). Mean relatedness for pairs for which parentage status could not be determined was intermediate (0.320 ± 0.135; N = 9), suggesting a mixture of parent-offspring and other combinations of adults and juveniles. Consistent with these findings, only 1 (33.3%) of the three parent-offspring pairs identified had an estimated r-value that was significantly less than 0.50 (**Fig.** 2). In contrast, 3 (75.0%) of the 4 pairs for which the adult was excluded as parent had r-values significantly less than 0.50; for adult-juvenile pairs of unknown parentage status, 4 (44.4%) of 9 pairs had r-values significant less than 0.50 (**Fig.** 2).

DISCUSSION

Our analyses revealed that in the population of *C. rionegrensis* at El Tabaré, multiple animals were captured at 15% of the burrow entrances at which trapping occurred. In addition to adults with juveniles, multiple adults –including adults of both sexes– were caught at several burrow entrances, indicating that the individuals captured together were not simply females and their dependent young. Indeed, as indicated by direct exclusion as well as parentage assignment analyses, the majority of adult-juvenile pairs captured together were not parent and offspring. Further, members of pairs that were identified as parent and offspring were captured at different burrow entrances, each typically with other animals that were not identified as first-order (r = 0.50) relatives. Consistent with this, animals captured together were not more closely related to each other than randomly selected pairs of individuals, providing no evidence that co-occurrence at a burrow entrance was associated with increased kinship. Collectively, these findings suggest that burrow systems in this species are occupied by larger, more complex sets of animals than would be expected in a strictly solitary species of subterranean rodent.

The data presented here reflect a relatively limited sampling of burrow use by *C. rionegrensis* and thus our findings are perhaps best interpreted as a preliminary depiction of the social structure of

Table 2

Microsatellite variability used to estimate genetic kinship in *C. rionegrensis*. For each of the 11 microsatellite loci examined, Ta is the annealing temperature used during PCR amplification of DNA samples. For each locus, the size of each allele (base pairs) is given, as is the relative frequency of each allele. Observed and expected heterozygosity for each locus are shown, as are the p-values for Hardy-Weinberg and linkage disequilibrium analyses for each locus. For HW tests, Bonferroni-corrected alpha = 0.005; for LD analyses, the corrected alpha = 0.003.

Locus	sizes (bp)	Allele frequency	Allele		H-W p-value
			Ho	He	
Soc 2	150	0.229	0.703	0.664	1.000
Ta=58°C	152	0.396			
	154	0.375			
Soc 3	129	0.021	0.857	0.554	0.512
Ta=59°C	222	0.292			
	133	0.083			
	135	0.604			
Soc 5	261	1.000	0.000	0.000	—-
Ta=62°C					
Soc 6	222	0.522	0.722	0.580	0.883
Ta=62°C	228	0.391			
	230	0.087			
Soc 7	280	0.174	0.647	0.51	0.186
(see text)	286	0.696			
	288	0.130			
Soc 8	154	0.208	0.500	0.337	1.000
(see text)	156	0.792			
Hai 3	163	0.479	0.769	0.510	0.584
Ta=68°C	165	0.521			
Hai 4	167	0.208	0.884	0.765	0.212
Ta=59°C	171	0.167			
	177	0.188			
	179	0.063			
	181	0.375			
Hai 9	228	0.188	0.545	0.438	0.178
Ta=59°C	232	0.063			
	234	0.729			
	238	0.021			
Hai 11	146	0.958	0.000	0.081	0.021
Ta=58°C	154	0.042			
Hai 12	112	0.022	0.296	0.165	1.000
Ta=60°C	114	0.065			
	122	0.913			

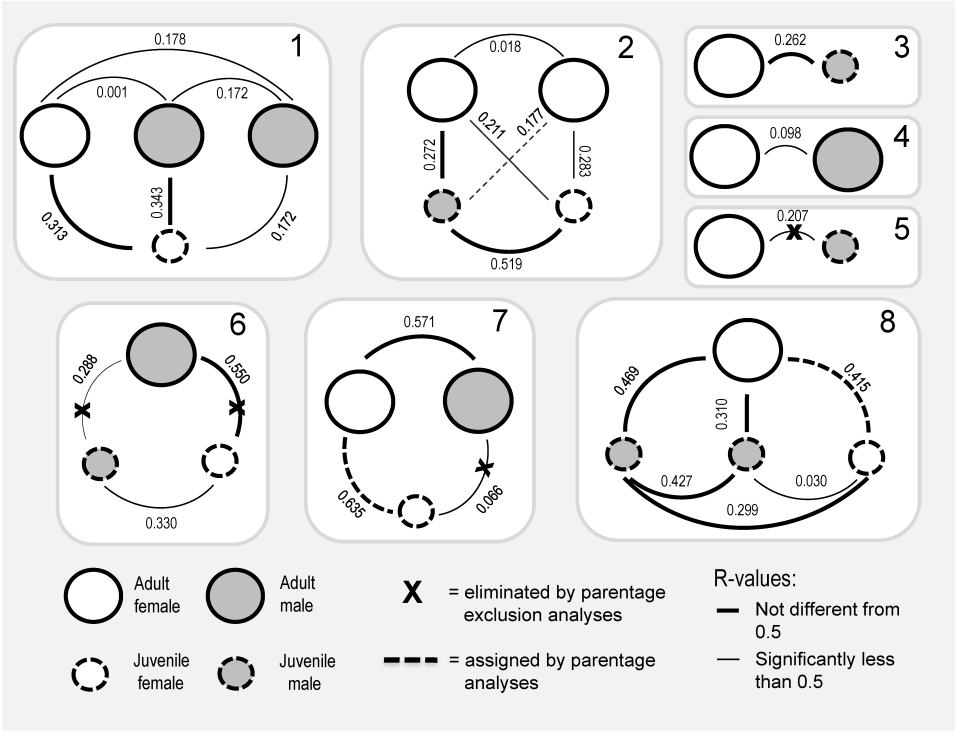

Fig. 2. Genetic estimates of kinship among individuals captured at the same burrow entrance. Pair-wise estimates of kinship were generated from microsatellite genotypes for 24 *C. rionegrensis* captured at 8 burrow entrances (**Fig.** 1). For each burrow entrance, the sexes and relative ages of the animals captured are indicated, as is the estimated coefficient of relatedness (r) for each pair of individuals. Narrow lines denote estimates of r that were significantly less than 0.50; wider lines denote estimates of r that did not differ from 0.5. Dashed lines indicate pairs for which the adult was assigned as the parent of that juvenile; pairs for which the adult was excluded as the parent of the juvenile are denoted with X's.

this species. Clearly, more extensive field sampling –including sampling of a larger number of burrow entrances over a longer portion of the year– would generate a more robust understanding of the behavior of the study population. In particular, use of radio-telemetry would allow real-time monitoring of the spatial distributions of individuals, thereby (1) providing more direct evidence of burrow sharing and (2) addressing concerns that multiple captures reflect immigration from neighboring burrow systems rather than actual sharing of burrows.

With regard to kinship, use of additional and more variable markers (e.g., single nucleotide polymorphisms or SNPs: Amorin & Pereira 2004; Hauser et al. 2011) should improve the resolution of analyses of kinship and parentage, thereby clarifying patterns of genetic relatedness among individuals occupying the same burrow system. Despite the limitations of the current study, however, our data clearly indicate that not all adults and juveniles captured together are parents and offspring. Thus, although further study of this system is required, our analyses provide compelling evidence that burrow systems are occupied by more than just a single adult female and her most recent litter of young.

Kin structure in ctenomyids

Coefficients of relatedness for adult *C. rionegrensis* captured at the same burrow entrance provided no indication that spatial overlap was associated with a specific pattern of kinship. Genetic estimates of kinship are available for only for one other ctenomyid, *C. talarum* (Cutrera et al. 2005). Although kinship in this solitary species tended to be greatest for neighboring females, pairwise estimates of relatedness did not generally differ from background levels of kinship for any combination of individuals, providing little support for intersexual differences in relatedness among spatially proximate adults (Cutrera et al. 2005). In contrast, demographic data indicate

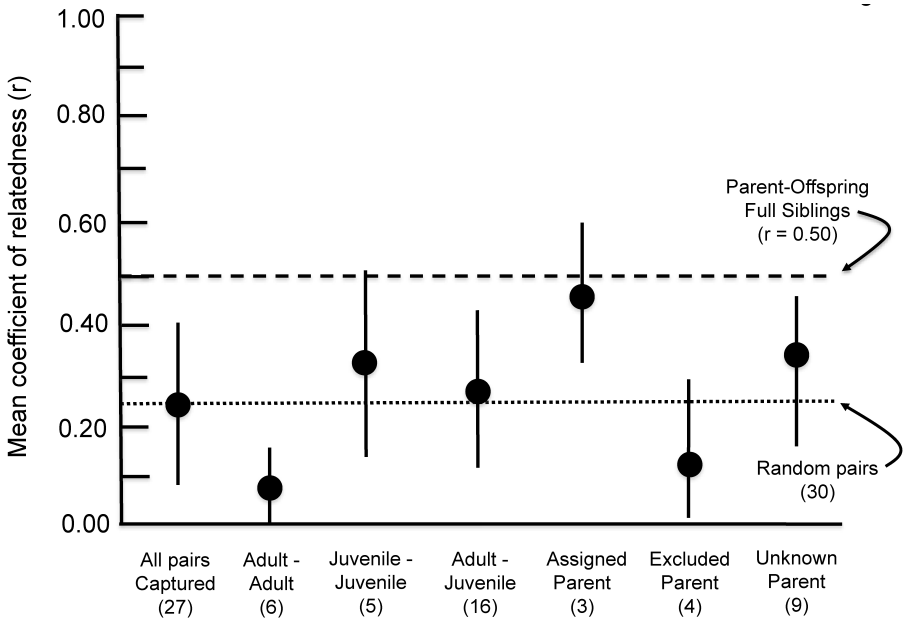

Fig. 3. Mean (± SD) values for coefficients of relatedness estimated from analyses of microsatellite loci; all estimates are based on pairwise comparisons of individual genotypes. The mean for all pairwise combinations of individuals captured at the same burrow entrance is shown, as is the mean for 30 randomly selected pairs of individuals (dotted line). For individuals captured together, mean values of kinship were also examined in relation to age class (e.g., adult-adult) and the results of genetic analyses of parentage (e.g., adult assigned or excluded as parent). The sample size for each comparison is given in parentheses.

that in the group-living *C. sociabilis*, female burrowmates are typically closely related to one another (e.g., sisters, mothers-daughters, aunt-niece; Lacey & Wieczorek 2004). Because all males of this species disperse from their natal burrow systems, they are not expected to be closely related to the females with which they share burrows as adults (Lacey & Wieczorek 2004). Thus, spatial patterns of kinship in *C. sociabilis* appear to vary markedly between the sexes. Our analyses suggest that *C. rionegrensis* is more similar to *C. talarum* in that kinship among our study animals did not vary detectably with the sexes of the animals captured together. Future studies of *C. rionegrensis* will explore the spatial structure of kinship in greater detail, including the extent to which kinship is influenced by patterns of natal dispersal by males and females of this species.

Implications for social structure

Our data indicating that burrow systems in the study population were used by multiple adults –including multiple adults of the same sex– are consistent with the findings of Tassino et al. (2011), who characterized patterns of burrow use in the same population of *C. rionegrensis* at Estancia El Tabaré. Using radiotelemetry, these authors demonstrated periodic overlap of home ranges for same- and opposite-sex adults, thereby providing evidence that burrow sharing was not limited to the animals included in our study. Although data were collected by Tassino et al. (2011) over a period of only 72 hours per field effort, sampling was repeated at three time points during the year, with spatial overlap of adults detected during two of these sampling periods. Thus, while the data presented by Tassino et al. (2011) are –like our findings– perhaps best viewed as preliminary, the outcomes of both studies are consistent in suggesting that burrow use in *C. rionegrensis* is not limited to a single adult.

The pattern of space use suggested here for *C. rionegrensis* differs from those reported for other species of ctenomyids studied to date. Radiotelemetry data from four species of tuco-tucos (*C. haigi*: Lacey et al. 1998, *C. talarum*: Cutrera et al. 2006, *C. australis*: Mora et al. 2010, *C. minutus*: Kubiak et al. 2017) indicate that these taxa are solitary, with no more than one adult captured per burrow system and no evidence of spatial overlap among

adults. In contrast, *C. sociabilis* is clearly group living, with burrow systems routinely occupied by multiple adult females, an adult male, and the associated litters of young; in this species, spatial overlap among animals captured together is persistent, extensive, and includes use of a shared nest site (Lacey et al. 1997; Lacey & Wieczorek 2004). The proposed pattern of space use in *C. rionegrensis* appears to fall somewhere between these extremes, with potentially regular but not continuous spatial overlap among adults. Although sample sizes are small, the overlap of home ranges reported by Tassino et al. (2011) as well as our data on multiple captures per burrow entrance indicate that these events are not limited to opposite-sex adults, suggesting that these relationships are not due solely to interactions between potential reproductive partners. Further analysis of the social structure of *C. rionengrensis*, including more extensive characterizations of spatial and kin relationships among individuals, will allow for more precise placement of this species within the growing comparative framework for ctenomyid social behavior.

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