Microsatellite variation in solitary and social tuco-tucos: molecular properties and population dynamics

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Variation at 15 microsatellite loci was characterized for a population of the solitary Patagonian tuco-tuco (*Ctenomys haigi*) and a population of group-living colonial tuco-tuco (*C. sociabilis*), both of which were located in the Limay River Valley of south western Argentina. All loci examined were characterized by uninterrupted di- or trinucleotide repeats in both species; seven of these loci had been isolated from *C. haigi* and eight had been isolated from *C. sociabilis*. Across all loci, there was a significant tendency for both number of alleles and heterozygosity to be greater in *C. haigi* than in *C. sociabilis*. Cloning and sequencing of multiple PCR products per locus per population revealed no significant biases in allele length, suggesting that this difference in variability was not due to ascertainment bias or to population-wide differences in rates of microsatellite evolution. Instead, differences in microsatellite variation between *C. haigi* and *C. sociabilis* were more consistent with current demographic and suspected historical differences between these populations. In particular, interpopulation differences in the distribution of microsatellite allele sizes and allele frequencies suggested that *C. sociabilis* has been more subject to historical reductions in population size (e.g. population bottlenecks) than has *C. haigi*. These findings indicate that analyses of microsatellite structure can yield important insights into the population-level phenomena that contribute to diversity at these markers, including differences in population history that continue to influence levels of genetic variability.

Keywords: *Ctenomys*, microsatellites, tuco-tucos, population bottleneck, variability.

Introduction

A fundamental goal of molecular studies of population structure is to determine how current and historical processes interact to shape patterns of genetic differentiation (Avise, 1994; p. 232). Comparative studies of closely related species that differ with respect to only one or a few key population parameters provide a potentially powerful means of achieving this objective. Inter-specific differences in genetic structure, however, may arise due to molecular-level differences among the taxa sampled as well as the population-level processes of interest. For example, as use of microsatellites to characterize population-level variability has increased, concerns have arisen regarding several aspects of microsatellite structure and evolution that may confound comparisons of genetic variation. These include differences in repeat motif (Estoup *et al.*, 1995; van

Treuren *et al.*, 1997), biases in allele lengths (Ellegren *et al.*, 1995), and changes in flanking-region sequences that produce non-amplifying alleles (Ishibashi *et al.*, 1996), each of which has been shown to influence levels of genetic variation detected at microsatellite loci. As a result, consideration of the molecular-level characteristics of the species sampled represents a critical component of comparative studies of genetic population structure.

As part of ongoing studies of the population biology of ctenomyid rodents, I examined patterns of microsatellite variation in the colonial tuco-tuco (*Ctenomys sociabilis*) and the Patagonian tuco-tuco (*C. haigi*). Although phylogenetic analyses based on sequence data from the cytochrome b region of the mitochondrial genome indicate that these species are not sister taxa (Lacey and Smith, in prep., but see also Lessa & Cook, 1998), both occur in the Limay River Valley in south-western Argentina, where populations of heterospecifics are separated only by the width of the Río Limay

(≈ 500 m). Despite their close physical proximity and their use of similar habitats, these animals exhibit striking differences in behaviour and demography. *C. haigi* is solitary, meaning that burrow systems of this species are never inhabited by more than a single adult (Lacey *et al.*, 1998). In contrast, burrow systems of *C. sociabilis* are routinely inhabited by multiple adult females and, in some cases, an adult male (Lacey *et al.*, 1997). Although more than 30 species of tuco-tucos are currently recognized (Woods, 1993), *C. sociabilis* is the only ctenomyid for which quantitative evidence of group living has been obtained and thus comparative studies of this species and *C. haigi* provide an apparently unique opportunity to examine the genetic consequences of interactions between behaviour demography and population history in ecologically similar tuco-tucos.

As a first step toward characterizing the genetic structures of *C. haigi* and *C. sociabilis*, I quantified microsatellite variation within one population of each species. The populations selected for analysis are the subjects of ongoing field research and thus their current behaviour and demography are well documented. Here, I report on the patterns of microsatellite variability evident in these populations and I assess the role of microsatellite structure in generating these differences. As these analyses indicate, rather than confounding comparative studies of genetic variability, the structural properties of microsatellite markers may yield important insights into the population-level processes underlying that variation.

Methods

Sample collection

The population of *C. haigi* sampled was located on Estancia San Ramon, Provincia Río Negro, Argentina ($40^{\circ}56'$ S, $71^{\circ}03'$ W). The population of *C. sociabilis* sampled was located directly across the Río Limay, on Estancia Rincon Grande, Provincia Neuquén, Argentina ($40^{\circ}56'$ S, $71^{\circ}03'$ W). During 1993–95, non-destructive tissue samples were collected from all animals trapped in these populations by removing the distal 2–3 mm of the outer digit on the right hind foot of each individual. Tissue samples were stored in DMSO₄-EDTA buffer (Seutin *et al.*, 1991) at ambient temperature. Because burrow sharing in *C. sociabilis* arises due to natal philopatry by females (Lacey, 2000), individuals from the same burrow system are typically closely related and are thus likely to share a substantial portion of their genomes due to common ancestry. To avoid underestimating genetic diversity in *C. sociabilis*, samples from 35 animals resident in 14 different burrow systems (2.6 ± 2.5 animals per system) were chosen for

analysis; the exact boundaries of these burrow systems had been determined by radiotelemetry as described in Lacey *et al.* (1997). In contrast, because related animals are not spatially clustered in *C. haigi*, samples from 35 randomly selected individuals resident throughout the study population were used to characterize microsatellite variation in this species.

Microsatellite analyses

Genetic variation at 16 microsatellite loci (later reduced to 15 loci; see results) was quantified for each study population. Eight of these loci had been isolated from *C. haigi* (Lacey *et al.*, 1999). The remaining eight loci were isolated from *C. sociabilis* following the methods of Lacey *et al.* (1999); these loci are described in Table 1. The 16 loci used in this study were selected because (1) they produced well-resolved PCR products for both *C. haigi* and *C. sociabilis* and (2) preliminary sequencing confirmed that each locus was characterized by a simple, uninterrupted repeat in both study species. None of these loci were screened to assess variability prior to the generation of the data reported here. A total of 35 adults per species (all conspecifics from the same local population) were screened using the eight loci isolated from *C. haigi*; a randomly selected subset of these individuals ($n = 20$ adults per population) were characterized using the eight microsatellite loci isolated from *C. sociabilis*.

To quantify microsatellite variability in each study population, high molecular weight genomic DNA was extracted from tissue samples via digestion with proteinase K, followed by phenol–chloroform extraction and ethanol precipitation (Sambrook *et al.*, 1989). PCR amplification of microsatellite loci was carried out in a 15 μ L volume containing 30 ng of DNA, 0.2 μ M each primer, 0.2 mM dNTP, 1 $\times$ Taq buffer (1.5 mM MgCl₂, 10 mM Tris-HCl, 50 mM KCl), and 0.75 units of Taq polymerase (Boehringer Mannheim). The thermocycling regime used consisted of 94°C for 5 min, followed by 34 cycles of 94°C for 30 s, Ta for 30 s, and 72°C for 45 s, with a final extension of 72°C for 5 min. Locus-specific annealing temperatures are given in Lacey *et al.* (1999) and in Table 1.

Allelic variability at each locus was examined by amplifying DNA as described above, but with one primer per pair end-labelled with [γ ³²P]-ATP via a kinase reaction (Richardson, 1965). Radioactively labelled PCR products (3 μ L per sample) were electrophoresed on 6% polyacrylamide, 40% urea denaturing sequencing gels at 1800 V for 2.5–6 h, as dictated by the size of the amplification products for each locus. Radioactive gels were then exposed to Kodak XAR film for 24–72 h at room temperature, -20°C , or -70°C . To determine the size in base pairs of each allele

Table 1 Characteristics of microsatellite loci isolated from *Ctenomys sociabilis*. T_a is the annealing temperature used in PCR reactions. Repeat motifs were sequenced from cloned PCR products, as described in the text. For each locus, the repeat motif for *C. sociabilis* is listed first, followed by the repeat motif for *C. haigi*. GenBank accession numbers are indicated

Locus	Primer sequences (5'–3')	T_a (°C)	Repeat motif	Genbank accession number
<i>Soc 1</i>	AACCTGAGGCACTGCATTCT GGAAACTTTGTCCCCAGTCT	58	(CA) ₁₃ (CA) ₁₅	AY007694
<i>Soc 2</i>	TGAGCTTCAACTTCCAGCAA AGTGTGTTTCTGCCACCTCC	58	(CA) ₁₄ (CA) ₁₂	AY007695
<i>Soc 3</i>	TGACCTACACAGTGAGACCCTG GACTTATGCCCTTTTGGCT	60	(CA) ₁₃ (CA) ₁₃	AY007696
<i>Soc 4</i>	CCCTGCACCAAAGAAATAAAA TGGGATGAGTTCTGTGTGGT	58	(CA) ₂₄ (CA) ₁₅	AY007697
<i>Soc 5</i>	GAAGGTTGGCAATGAGCAGAT TGCAGCTAAAAGGTGAAGGC	62	(CA) ₂₀ (CA) ₂₅	AY007698
<i>Soc 6</i>	CAAGCTCTTGCCCATCACTT ACTGCAGCTCAGGGGTAGTG	62	(CA) ₂₁ (CA) ₁₆	AY007699
<i>Soc 7</i>	CCCCCACTGAAAGCGTTAT GACTGCTGGCAACTCAGGTT	60	(CA) ₁₄ (CA) ₁₂	AY007700
<i>Soc 8</i>	TGCAGTAAGCAGAGGCTGAA TTGACAGGAAGAATCTGGGG	60	(CA) ₁₉ (CA) ₁₇	AY007701

detected, radioactively labelled M13mp18 sequence was used as a size standard on all gels. In addition, amplicons from two to three individuals per locus were run on all gels to facilitate consistent scoring of microsatellite alleles.

To determine the number of repeat units associated with each allele, amplicons of several alleles per locus were cloned (TA Cloning Kit, Invitrogen) and three to five positive bacterial colonies from each cloning reaction were sequenced (T7 promoter primer, USB) on an ABI 377 automated sequencer. For monomorphic loci, sequences from at least two individuals were compared to confirm that the number of repeat units assigned to the single allele detected was consistent. For variable loci, 2–3 alleles per locus were sequenced; the results of sequencing and radioactive screening analyses were compared to determine if size differences among alleles detected via comparison with the M13 sequence were consistent with differences in the number of repeat units assigned to distinct alleles.

Data analysis

Allelic variation at each locus was scored manually by aligning amplified fragments with the M13 size standard and the 'reference' individuals included on all gels. Genotypes were assigned to individuals based on the presence of one (homozygous) or two (heterozygous) distinct bands at each locus screened. Observed allele frequencies for each study population were calculated

and were compared to values expected under Hardy–Weinberg conditions using ARLEQUIN 1.1 (Schneider *et al.*, 1995). Analyses of linkage disequilibrium were also performed using ARLEQUIN 1.1. All other statistical procedures were performed using STATISTICA 5.0 (Statsoft, 1997); because several aspects of the microsatellite data (e.g. number of alleles per locus) were not normally distributed, nonparametric tests were used for most analyses. Graphical analyses of allele frequency distributions used to detect recent bottlenecks in the study populations were completed as described in Luikart *et al.* (1998). To quantitatively compare the likelihood of historical bottlenecks in the two study populations, estimates of M (mean ratio of number of alleles to range of allele sizes) were generated according to Garza & Williamson (2001). Throughout the text, means are reported ± 1 standard deviation.

Results

Population-level variability

Levels of microsatellite variability differed markedly among the study populations in all three years for which samples were analysed. Although an individual's genotype was represented only once in the molecular data set, many of the animals sampled were present in the focal populations during more than one year of the study, suggesting that genotypic data from 1993, 1994 and 1995 could be pooled to yield a single, larger data

set. The limited number of animals screened each year ($n \leq 15$) precluded the use of standard homogeneity tests (e.g. Weir, 1990) to determine if allele frequencies differed across years. Instead, because allelic variants were identified on the basis of size variation in PCR products, mean allele sizes (in base pairs) at each locus were compared across years using one-way analyses of variance. No significant differences in mean allele sizes were detected (all $P > 0.050$) for either population and thus data for 1993–95 were pooled for further analysis.

When each locus was considered independently, allele frequencies at two loci in the pooled data set (*Soc 1* and *Soc 2*) deviated significantly from Hardy–Weinberg expectations in *C. haigi* (Table 2). When a Bonferroni correction (Rice, 1989) was applied to these analyses, however, no significant departures from Hardy–Weinberg expectations were detected (corrected $\alpha = 0.0016$, $P > 0.002$; Table 2). Pairwise comparisons of allele frequencies for *C. haigi* revealed significant linkage disequilibrium among two loci (*Hai 2* and *Hai 3*; Bonferroni-corrected $\alpha = 0.0004$, $P < 0.0001$) and thus locus *Hai 2* was excluded from further consideration, reducing to 15 the number of loci used in subsequent analyses. Low levels of allelic variability in *C. sociabilis* (Table 2) precluded most pairwise assessments of linkage disequilibrium and thus I assumed that patterns of linkage in this species were the same as those in *C. haigi*.

All of the 15 loci included in subsequent analyses were variable in *C. haigi*. In contrast, eight (53.3%) of these loci were monomorphic in *C. sociabilis* (Table 2). Of the seven loci that were variable in *C. sociabilis*, two (28.6%) had been isolated from *C. haigi*; the remaining five (71.4%) were isolated from *C. sociabilis*. The occurrence of variable loci in *C. sociabilis* was not significantly associated with the species of origin for the microsatellite regions examined (Fisher's Exact Test, two-tailed $P > 0.050$). The number of alleles per locus detected for *C. sociabilis* ranged from one to three. In contrast, the number of alleles per locus detected for *C. haigi* ranged from three to 13. For all loci, the number of alleles detected was greater in *C. haigi* than in *C. sociabilis* (Fig. 1a); this tendency was significant (Wilcoxon Signed Rank Test, $T = 0.001$, $n = 15$, $P < 0.001$). Similarly, for all loci, observed heterozygosity was greater in *C. haigi* than in *C. sociabilis* (Fig. 1b); again, this tendency was significant (Wilcoxon Signed Rank Test, $T = 0.001$, $n = 15$, $P < 0.001$).

Analyses of allele structure

For all loci, intrapopulation differences in allele size were consistent with variation due to changes in the number of repeat units in the microsatellite region. Within each population, allele lengths always varied by

multiples of the core repeat sequence (2 or 3 bp) and, at a given locus, differences among the number of repeat units in cloned alleles were consistent with the total length differences among PCR products for those alleles. Across populations, flanking sequences amplified by the same primer pair were extremely similar. For six of the loci analysed, flanking sequences for *C. sociabilis* and *C. haigi* were identical. For the remaining loci examined, insertions–deletions of 1–7 base pairs (mean = 2.7 ± 1.7 bp, $n = 18$ indels) were evident between populations. Seven (38.9%) of these indels were located immediately adjacent to repeat regions, with the rest scattered throughout the surrounding sequence. The mean percentage sequence divergence between *C. sociabilis* and *C. haigi* due to indels in the flanking regions of a locus was $2.7 \pm 2.6\%$ ($n = 15$ pairs of sequences).

Analyses of allele size

Within each population, there was no significant difference between the mean number of repeat units at loci isolated from *C. haigi* and those isolated from *C. sociabilis* (*C. haigi*: Mann–Whitney $U = 26.0$, $n = 7, 8$, $P > 0.050$; *C. sociabilis*: Mann–Whitney $U = 27.5$, $n = 7, 8$, $P > 0.050$); as a result, data from all microsatellite loci were combined for the following analyses of allele size variation. In this combined data set, there was no significant tendency for the mean number of repeat units at a locus to be greater in one study population than in the other (Wilcoxon Signed Rank $T = 44.5$, $n = 15$, $P > 0.050$). Similarly, there was no significant tendency toward a greater mean number of repeat units in either population when comparisons were restricted to loci that were monomorphic in *C. sociabilis* (Wilcoxon Signed Rank $T = 14.0$, $n = 8$, $P > 0.050$). Within *C. sociabilis*, the mean number of repeat units at monomorphic loci was not significantly different from that at variable loci (Mann–Whitney $U = 27.5$, $n = 7, 8$, $P > 0.050$), indicating that the absence of multiple alleles was not due to particularly short repeat regions at monomorphic loci.

To examine patterns of allele size variation in greater detail, the mean pairwise difference in number of repeat units between alleles was determined for each locus; for simplicity, this measure is referred to as the mean size difference among alleles. Mean size differences among alleles for *C. haigi* ranged from 1.3 to 6.5 repeat units per locus; across loci, mean allele size differences for this population were normally distributed (Fig. 2; Kolmogorov–Smirnov $d = 0.100$, $P > 0.050$). In contrast, mean allele size differences for *C. sociabilis* ranged from 0 to 9 repeat units per locus and, across loci, mean size differences appeared almost bimodal in distribution; the majority (66.7%) of the 15 loci examined exhibited

Table 2 Summary of microsatellite variation in *Ctenomys sociabilis* and *C. haigi*. Data are from one population per species. A total of 35 adults per species were screened for loci isolated from *C. haigi* (Hai 2–Hai 12); a total of 20 adults per population were screened for loci isolated from *C. sociabilis* (Soc 1–Soc 8). The number of alleles per locus is reported, as are the frequencies of all alleles (PCR product size for each allele reported in parentheses). Observed and expected heterozygosities are indicated, as are the results of Hardy–Weinberg analyses

Locus	Species	No. of alleles	Allele frequencies (PCR product sizes in bp)	Ho	He	Hardy–Weinberg <i>P</i> -value†
Hai 2	<i>sociabilis</i>	1	1.000 (132)	0.000	0.000	—
	<i>haigi</i>	8	0.229 (140), 0.271 (146), 0.214 (148), 0.129 (152), 0.086 (144), 0.026 (154), 0.026 (134), 0.014 (150)	0.771	0.803	0.529
Hai 3	<i>sociabilis</i>	1	1.000 (134)	0.000	0.000	—
	<i>haigi</i>	9	0.297 (130), 0.257 (128), 0.122 (120), 0.081 (124), 0.081 (126), 0.068 (132), 0.041 (134), 0.027 (116), 0.027 (118)	0.838	0.808	0.943
Hai 4	<i>sociabilis</i>	3	0.971 (140), 0.014 (138), 0.014 (142)	0.057	0.057	1.000
	<i>haigi</i>	13	0.243 (126), 0.203 (128), 0.203 (132), 0.108 (124), 0.081 (138), 0.041 (118), 0.027 (134), 0.027 (136), 0.014 (130), 0.014 (140), 0.014 (142), 0.014 (144), 0.014 (146)	0.784	0.836	0.385
Hai 5	<i>sociabilis</i>	1	1.000 (155)	0.000	0.000	—
	<i>haigi</i>	6	0.689 (159), 0.216 (165), 0.054 (169), 0.014 (161), 0.014 (163), 0.014 (167)	0.459	0.473	0.200
Hai 8	<i>sociabilis</i>	1	1.000 (95)	0.000	0.000	—
	<i>haigi</i>	10	0.557 (85), 0.100 (107), 0.086 (113), 0.071 (99), 0.057 (97), 0.029 (87), 0.029 (93), 0.029 (105), 0.029 (109), 0.014 (117)	0.629	0.661	0.156
Hai 9	<i>sociabilis</i>	1	1.000 (181)	0.000	0.000	—
	<i>haigi</i>	3	0.569 (173), 0.417 (175), 0.014 (177)	0.528	0.502	1.000
Hai 11	<i>sociabilis</i>	2	0.914 (140), 0.086 (142)	0.171	0.158	1.000
	<i>haigi</i>	11	0.221 (152), 0.172 (140), 0.132 (132), 0.088 (138), 0.088 (154), 0.074 (134), 0.074 (142), 0.059 (136), 0.059 (148), 0.029 (150), 0.015 (144)	0.941	0.879	0.846
Hai 12	<i>sociabilis</i>	1	1.000 (92)	0.000	0.000	—
	<i>haigi</i>	7	0.342 (95), 0.250 (92), 0.158 (86), 0.118 (89), 0.066 (83), 0.053 (98), 0.013 (101)	0.842	0.795	0.931
Soc 1	<i>sociabilis</i>	2	0.900 (217), 0.100 (229)	0.200	0.190	1.000
	<i>haigi</i>	7	0.263 (229), 0.211 (227), 0.184 (235), 0.158 (225), 0.079 (233), 0.053 (231), 0.053 (239)	0.842	0.815	0.046
Soc 2	<i>sociabilis</i>	1	1.000 (93)	0.000	0.000	—
	<i>haigi</i>	9	0.500 (107), 0.105 (103), 0.079 (93), 0.079 (109), 0.052 (89), 0.052 (91), 0.052 (95), 0.052 (113), 0.026 (111)	0.421	0.714	0.002
Soc 3	<i>sociabilis</i>	1	1.000 (80)	0.000	0.000	—
	<i>haigi</i>	3	0.763 (78), 0.158 (80), 0.079 (92)	0.263	0.387	0.085

Table 2 (Continued)

Locus	Species	No. of alleles	Allele frequencies (PCR product sizes in bp)	Ho	He	Hardy–Weinberg <i>P</i> -value†
<i>Soc 4</i>	<i>sociabilis</i>	3	0.900 (364), 0.050 (354), 0.050 (370)	0.100	0.184	0.052
	<i>haigi</i>	5	0.421 (354), 0.395 (352), 0.105 (358), 0.053 (350), 0.026 (356)	0.631	0.652	0.376
<i>Soc 5</i>	<i>sociabilis</i>	2	0.875 (232), 0.125 (250)	0.219	0.218	1.000
	<i>haigi</i>	6	0.526 (218), 0.158 (240), 0.158 (244), 0.079 (238), 0.053 (236), 0.026 (234)	0.684	0.666	0.851
<i>Soc 6</i>	<i>sociabilis</i>	2	0.850 (195), 0.150 (181)	0.300	0.254	1.000
	<i>haigi</i>	11	0.447 (185), 0.158 (175), 0.079 (193), 0.079 (197), 0.053 (179), 0.053 (189), 0.026 (181), 0.026 (183), 0.026 (187), 0.026 (191), 0.026 (195)	0.895	0.752	0.823
<i>Soc 7</i>	<i>sociabilis</i>	2	0.900 (224), 0.100 (236)	0.200	0.180	1.000
	<i>haigi</i>	7	0.316 (237), 0.289 (231), 0.132 (239), 0.105 (225), 0.105 (241), 0.026 (243), 0.026 (245)	0.737	0.775	0.270
<i>Soc 8</i>	<i>sociabilis</i>	1	1.000 (114)	0.000	0.000	—
	<i>haigi</i>	5	0.289 (109), 0.237 (115), 0.237 (117), 0.132 (113), 0.105 (111)	0.737	0.776	0.791

†*P*-values for each locus are shown. When a sequential Bonferroni correction (Rice, 1989) is applied to these analyses ($k = 32$), no significant deviations from Hardy–Weinberg expectations are evident.

mean size differences of 0–1 repeat units, with the remaining loci exhibiting mean size differences of ≥ 5 repeat units (Fig. 2). Although this distribution was not significantly different from normal (Kolmogorov–Smirnov $d = 0.328$, $0.050 < P < 0.080$), visual inspection of Fig. 2 suggests that patterns of mean size difference among alleles varied markedly among the two study populations.

For *C. sociabilis*, all loci with mean allele size differences of greater than four repeat units (Fig. 2) had been isolated from this species; this association between mean allele size difference and species of origin was significant (Fisher's Exact Test, two-tailed $P < 0.040$). In contrast, comparable mean differences in allele size in *C. haigi* were not significantly associated with species of origin (Fisher's Exact Test, two-tailed $P > 0.050$). Thus, in the study population of *C. sociabilis*, variable loci isolated from *C. haigi* were characterized by alleles that differed in size by only one or two repeat units, whereas variable loci isolated from *C. sociabilis* were characterized by alleles that, on average, differed in size by four or more repeat units.

Allele frequency distributions

Histograms of allele frequencies (all loci combined) differed markedly between the study populations. The distribution of allele frequencies for *C. haigi* was

L-shaped, with greater than 60% of the 112 alleles detected occurring at frequencies of ≤ 0.10 (Fig. 3). Based on the criteria established by Luikart *et al.* (1998), this distribution suggests that the study population of *C. haigi* has not been subject to a recent bottleneck event. In contrast, the distribution of allele frequencies for *C. sociabilis* was much more bimodal; while roughly one-third of the 24 alleles detected occurred at frequencies of ≤ 0.10 , 42% of these alleles occurred at frequencies of > 0.90 (Fig. 3). This distribution does not strictly conform to the criteria for detecting bottlenecks established by Luikart *et al.* (1998) in that alleles of intermediate frequencies (0.11–0.90) are underrepresented. Nevertheless, the distribution of allele frequencies in the study population of *C. sociabilis* is clearly shifted toward a smaller number of more abundant alleles, as expected in populations that have experienced a bottleneck.

Estimates of *M*, the mean ratio of number of alleles to range of allele sizes (measured as number of repeat units), differed markedly between the study populations. For the seven loci that were variable in both study populations, *M* for *C. sociabilis* was 0.48; in contrast, *M* for *C. haigi* was 0.81. Garza & Williamson (2001) assert that, for data sets based on ≥ 7 loci, values of $M \leq 0.68$ are indicative of a recent population bottleneck. Use of this criterion indicates that whereas the study population of *C. sociabilis* has experienced a

Fig. 1 Observed (a) number of alleles and (b) heterozygosity at 15 microsatellite loci from tuco-tucos. In both panels, data from *Ctenomys haigi* (grey bars) are presented above the horizontal axis; data from the homologous loci in *C. sociabilis* (black bars) are presented below this axis. The first seven loci depicted were isolated from *C. haigi*; a total of 35 adults per population were screened for variability at these loci. The remaining eight loci were isolated from *C. sociabilis*; a total of 20 adults per population were screened at these loci.

recent bottleneck event, the study population of *C. haigi* has not. Thus, both graphical analyses of allele frequency distributions and calculations of M suggest that *C. sociabilis* has been more subject to historical reductions in population size than has *C. haigi*.

Discussion

Levels of microsatellite variability differed markedly among the study populations. At all loci, both the number of alleles detected and the observed heterozygosity were greater in the solitary *C. haigi* than in the group-living *C. sociabilis* (Fig. 1). This tendency was evident regardless of the species from which repeat regions had been isolated. In addition to these differ-

Fig. 2 Histogram of mean size differences among alleles at 15 microsatellite loci examined in *Ctenomys sociabilis* and *C. haigi*. Data on allele sizes (measured in number of repeat units) were obtained by cloning and sequencing PCR products from multiple individuals per locus per population. For each locus, the mean pair-wise size difference among alleles was determined for each study population; these values were rounded to the nearest integer and the number of loci per mean allele size was summed.

ences in variability, the study populations exhibited pronounced differences in patterns of allele size variation. In *C. haigi*, mean differences in allele size were normally distributed (Fig. 2), while in *C. sociabilis*, mean differences in allele size were nearly bimodal in distribution (Fig. 2). Mean size differences among alleles were not related to species of origin in *C. haigi*; in contrast, in *C. sociabilis*, such differences were significantly associated with the species from which microsatellites had been isolated. The distribution of allele frequencies also differed among the study populations, with allele frequencies for *C. sociabilis* clearly shifted toward the presence of a limited number of very common alleles (Fig. 3). Collectively, these data suggest intriguing differences in the processes underlying the maintenance of microsatellite variation in the two study populations.

Multiple factors may have contributed to the differences in microsatellite variability evident between the study populations, including differences in current behaviour and demography, population history and molecular properties.

Fig. 3 Comparison of allele frequency distributions in the two study populations. The proportion of all alleles detected (*Ctenomys haigi*, $n = 112$; *C. sociabilis*, $n = 24$) falling within each allele frequency class is shown for each population. Interpretations of these distributions based on Luikart *et al.* (1998) indicate that the allele frequency distribution for *C. haigi* is consistent with that expected for populations that have not experienced a recent reduction in size; in contrast, the distribution of *C. sociabilis* more closely resembles those from populations known to have experienced recent bottleneck events.

Differences in current behaviour and demography

As already noted, *C. haigi* and *C. sociabilis* exhibit a number of behavioural and demographic differences that are expected to impact patterns of population-level genetic variation in these species. The most conspicuous of these differences is the formation of social groups by *C. sociabilis*. Group living in this species arises due to natal philopatry by females (Lacey *et al.*, 1997; Lacey, 2000), which leads to the spatial aggregation of individuals that share alleles due to recent common ancestry. The presence of distinct social units is typically expected to favour increased genetic variation within local populations due to the maintenance of genetic differences among groups (Dobson *et al.*, 1998). In *C. sociabilis*, however, rapid turnover of groups and recolonization of burrow systems from within the population, combined with limited migration between populations (Lacey, unpublished data), suggest that all members of a local population share a considerable portion of their genetic material due to shared ancestry.

In contrast, burrow systems of *C. haigi* are inhabited by only a single adult (Lacey *et al.*, 1998), with no tendency for related individuals (e.g. females and offspring) to occupy adjacent burrow systems (Lacey, unpublished data). These behavioural differences, com-

bined with the tendency for *C. haigi* burrows to be recolonized by individuals from outside the study population (Lacey, unpublished data), imply that variation within local populations of this species should be greater than that within local populations of *C. sociabilis*. The microsatellite data reported here are consistent with this expectation, suggesting that current behavioural and demographic variables contribute to the differences in genetic variability evident among the study populations.

Differences in population history

Historical differences between the study populations may also contribute to the patterns of microsatellite variation reported here. In particular, interspecific differences in patterns of population formation and the frequency of population bottlenecks may play a role in generating the observed differences in genetic variability. Populations that are founded by a small number of individuals will have relatively low levels of initial variability (i.e. a strong founder effect); this limited variability may be perpetuated due to subsequent low levels of gene flow and consistently small effective population size (Hartl & Clark, 1997). Populations that are subject to pronounced bottlenecks will undergo marked reductions in variability, the effects of which may remain evident in current surveys of genetic variation (Avice, 1994).

Although the histories of the study populations are not known, the current demographic differences between these populations suggest that they are likely to have experienced different historical rates of gene flow, drift, and extinction/recolonization. In addition, the distinct allele size and allele frequency distributions reported here are consistent with differences in the frequency of population bottlenecks. The distribution of mean allele size differences in *C. sociabilis*, in particular the presence at several loci of a limited number of alleles of very different sizes (Fig. 2), is suggestive of one or more historical bottlenecks in which only a few alleles of quite different sizes were preserved (e.g. Garza & Williamson, in press). Further, whereas the distribution of allele frequencies for *C. haigi* is consistent with that expected for non-bottlenecked populations, the distribution of allele frequencies for *C. sociabilis* is more consistent with patterns observed in populations thought to have experienced a recent bottleneck event (Luikart *et al.*, 1998). Finally, estimated M for *C. sociabilis* falls within the range of values thought to reflect recently bottlenecked populations, whereas M for *C. haigi* does not. If these interpretations of the allele size and allele frequency data are correct, they suggest that the study population of *C. sociabilis* has been more subject to past reduction(s) in population size than has

the study population of *C. haigi*. Thus population history may contribute not only to the observed difference in levels of microsatellite variability, but also to the patterns of allelic variation underlying this difference in variability.

Differences in molecular properties

In addition to the population-level parameters discussed above, several structural attributes of microsatellite markers may contribute to the observed differences in patterns of genetic variation. In the present study, multiple steps were undertaken to reduce the probability that the genetic data were influenced by such factors. First, only microsatellites characterized by the same repeat motif in both study populations were screened (Table 1; Lacey *et al.*, 1999); by controlling for differences in repeat motif and by restricting comparisons almost exclusively to dinucleotide repeats, motif-related biases in variability (e.g. Pépin *et al.*, 1995; Chakraborty *et al.*, 1997) should have been minimized. Second, none of the loci included in this study were screened to assess variability prior to the generation of the data summarized in Table 2. As a result, measures of variability at these loci should not have been subject to ascertainment biases associated with the selection of a particular subset of loci for analysis (e.g. the most variable loci in one species; Ellegren *et al.*, 1995). Indeed, because loci isolated from both *C. sociabilis* and *C. haigi* were used, the consistently greater variability detected for *C. haigi* cannot be attributed to structural or other properties of microsatellites that differ between source and non-source species for these markers.

With regard to the data generated, observed heterozygosities at variable loci in both study populations were consistent with Hardy–Weinberg expectations (Table 2), providing no evidence that these data were influenced by the presence of null alleles (Pemberton *et al.*, 1995). Although Hardy–Weinberg calculations could not be used to assess the potential for null alleles at the eight loci that were monomorphic for *C. sociabilis*, the marked similarity of flanking region sequences across the study species suggests that non-amplification of alleles due to primer–template mismatch was unlikely. It also seems unlikely that non-amplification of alleles would result in monomorphism at eight apparently unlinked loci in *C. sociabilis* (Table 2), including three loci isolated from that species. Thus, non-amplification of alleles does not appear to provide a comprehensive explanation for the consistently greater microsatellite variability evident in *C. haigi*.

The one aspect of microsatellite behaviour that did appear to vary between the study populations was the distribution of allele sizes at a locus. As suggested by

Fig. 2, whereas mean allele size differences for *C. haigi* were normally distributed, mean size differences for *C. sociabilis* appeared to be almost bimodal in distribution, with some loci characterized by very pronounced differences in allele size. Although this association between allele size difference and species of origin at first appears suggestive of some form of ascertainment bias, the absence of any other interspecific biases in allele size, structure, or behaviour provides no obvious mechanism for generating this relationship. Instead, given the limited number of variable loci detected in *C. sociabilis*, it is possible that this relationship is simply a stochastic consequence of the microsatellite regions chosen for analysis.

Conclusions

Collectively, these analyses suggest that the striking differences in variability evident between the study populations reflect population-level, rather than molecular-level processes. Specifically, the absence of detectable biases in microsatellite structure, combined with the greater polymorphism and heterozygosity evident in *C. haigi* across all loci, suggests that variability in the study populations is influenced primarily by current demography and population history. In particular, the distributions of allele size differences and allele frequencies in *C. sociabilis* suggest that this population has been more subject to historical reductions in population size than has the study population of *C. haigi*. Thus, investigation of the structure and behaviour of the microsatellite markers used in this study not only mitigates concerns regarding potentially confounding molecular-level phenomena, but also provides intriguing suggestions as to the nature of the population-level forces that have contributed to markedly different levels of variability in the study populations. Future studies of these animals will explore the interplay of these factors in greater detail by using the microsatellite markers characterized here to assess patterns of among-population variation in each study species.

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