

Mitochondrial variation of a Belding's ground squirrel (*Spermophilus beldingi*) population



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Abstract:

Social behavior can impact evolutionary processes. Therefore, given the opportunity to examine a species exhibiting high levels of social interaction enables us to understand the connection between social behavior and genetic structure. In particular, we are interested in Belding's ground squirrels (*Spermophilus beldingi*) whose social behavior is distinctly characterized by nepotism. Although this particular species is not under any present threat it may be used as model for conservation efforts to understand other social species at risk of extinction. As part of a preliminary study, observations of individuals were made for the identification of mothers and offspring from one population. DNA was then isolated from these individuals to be used for genetic analyses. Portions of the mitochondrial DNA from the cytochrome-*b* gene (*cyt-b*) and the displacement loop (D-loop) were amplified using PCR and then sequenced. Approximately 400 base pairs from each region were examined to assess within-population haplotype diversity.



Fig. 2 Study site, 20 hectare meadow near the Tioga Pass in Mono County, CA

Fig.1 *S. beldingi* individual



Introduction:

Previous findings suggest that populations of Belding's ground squirrels are healthy and have high numbers (Nunes et al.) whereas other closely related species (i.e. *S. bunneus* and *S. mollis*) are threatened with extinction (Antolin et al. 2001, Sherman & Runge 2002). Thus, we chose to examine one particular population of Belding's ground squirrels in Mono County, which has been studied for behavioral purposes over several years, as a model for conservation purposes. By looking at genetic variation within this population we can infer the level of inbreeding, occurrences of bottlenecks, and other related processes. In this study, molecular diversity was analyzed using regions of the mitochondrial DNA (mtDNA): the cytochrome-*b* gene (*Cyt-b*) and the displacement loop (D-loop). These markers were chosen due to their matrilineal mode of inheritance, rapid rate of evolution, and the ease of determining haplotype (combinations of alleles of different genes on the same chromosomal homolog) lineages in the absence of severe gene flow (Arbogast 1999). Additionally, observational data was combined with molecular data, providing us with a model to better understand intrapopulation variation.

Materials & Methods:

Sample Collection

- behavioral observations were made for a two year period during breeding seasons
- squirrels were trapped at the study site (Fig. 2) using Tomahawk live traps
- a small portion of 1 ear was clipped from each individual and preserved in 1ml tubes
- each individual was marked with a numbered tag placed in the other ear
- DNA was extracted using QiAamp DNA Mini Kit and frozen

Amplification/sequencing *Cyt-b* & D-loop regions

- amplified 460 bp segment of *Cyt-b* with primers: L14115 & H15228
- amplified 360 bp segment of control region of 5 individuals with primers: L15372-MTL3 & H41_MTL2
- purified PCR products using QIAquick PCR Purification Kit
- sequencing reactions performed at the UCSF Biomolecular Resource Center
- contiged and edited sequences in Sequencher® (Fig. 4)
- gene diversity and haplotype frequencies were estimated using Arlequin 2.0



Fig. 3 Removal of ear tissue for DNA sample

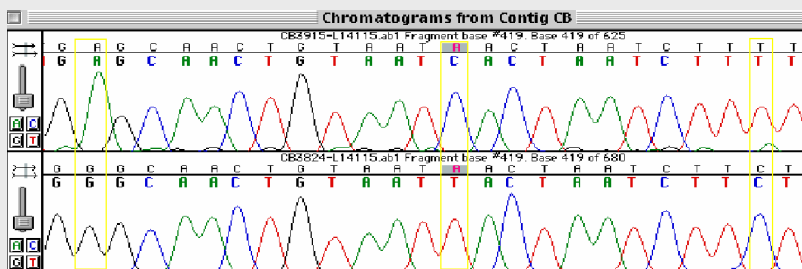


Fig. 4 Chromatogram illustrating 3 polymorphic sites located in the *Cyt-b* region

Results:

Cyt-b Region:

- ✓ 19 individuals sequenced
- ✓ 5 polymorphic sites observed
- ✓ # of observed transitions: 5
- ✓ # of observed transversions: 0
- ✓ Gene diversity: $h = 0.6082$

Haplotype Frequencies:

AGTCCT = 0.526
GACTCC = 0.368
GGTCCT = 0.105

D-loop Region:

- ✓ 5 individuals sequenced
- ✓ 1 haplotype observed, no polymorphic sites

Discussion:

Our preliminary study suggests that this *S. beldingi* population is characterized by a moderate level of genetic diversity $h = 0.61$, indicating that inbreeding is not a problem. Within the population three *Cyt-b* haplotypes were identified with no pronounced sex differences. As expected, mother's and siblings shared identical haplotypes. As shown in previous research, the results indicate that the *Cyt-b* gene can be used as molecular marker to determine baseline genetic structure. We were only able to sequence the D-loop region for five individuals, and no variation was observed. However, half of these individuals exhibited polymorphism for the *Cyt-b* region, suggesting this region of the D-loop may not be as variable as the *Cyt-b* region. Thus, additional sampling needs to be completed to accurately compare D-loop with *Cyt-b* variation. Moreover, future comparisons must be made with other populations to determine dispersal and gene flow patterns. Concurrently, nuclear markers such as microsatellites can serve as a useful tool to examine paternity and other existent sources of population gene flow. We hope to incorporate molecular data (mtDNA and microsatellites) with behavioral observations. The correlation between the genetic and behavioral data may lead to a greater understanding of dispersal patterns and genetic structure for this particular ground squirrel population. Furthermore, for conservation purposes, this type of information may be valuable for managing threatened squirrel populations.