

of significance, our DPS policy suggests that we consider whether there is evidence that the population represents the only surviving natural occurrence of a taxon that may be more abundant elsewhere as an introduced population outside its historic range. The western gray squirrel in Washington is not the only surviving natural occurrence of the subspecies. Consequently, this factor is not applicable to our determination regarding significance.

**Marked Differences in Genetic Characteristics.** The DPS policy suggests that one measure of significance is evidence that the discrete population segment differs markedly from other populations of the subspecies in its genetic characteristics. Preliminary evidence of genetic variation among the three western gray squirrel populations in Washington and two populations in Oregon showed that genetic variability may exist (Parametrix, Inc. 1999). The sample sizes, however, were too small for substantive conclusions (M. Linders, pers. comm. 2003d).

In 2003, researchers from WDFW and the University of Washington completed genetic analyses, using standard conservation genetic research techniques, the results point towards significant genotypic differentiation between Washington populations and squirrel populations south of the Columbia River. The report presents the results of two different types of genetic analyses (microsatellite DNA analysis and mitochondrial control region sequence analyses). The following discussion of the results of the genetic analyses is summarized from Warheit (2003).

#### *Microsatellite DNA Analysis*

Microsatellite DNA analyses were completed on samples from 128 western gray squirrels from California (3), Oregon (24), and Washington (101). Samples were obtained from museum skins, museum tissue collections, road-killed individuals, and ear punches from live-trapped individuals.

Microsatellites are short (no longer than six base pairs (nitrogenous bases that are part of the DNA molecule, such as cytosine and guanine)) tandemly repeated segments interspersed throughout the chromosome. Changes in the repeats result in different lengths of DNA, and a specific length of DNA can be used as a marker for a microsatellite locus (position on the chromosome). Seven of these loci that showed variation were analyzed. The results of the microsatellite analysis was summarized by the genetic diversity (the variation in chromosomes) and the

genetic differentiation (how different genetically are the populations).

#### *Genetic Diversity*

- An allele is a series of two or more different genes that occupy the same position on a chromosome. All populations in Oregon and California showed at least three private alleles (alleles present in only that population), while no Washington population had a private allele. This indicates that while all alleles present in each of the Washington populations are also present in at least one of the Oregon or California populations, there are alleles present in either Oregon or California that are not present in Washington.
- The Washington populations show reduced genetic diversity at all measures compared with populations south of the Columbia River, despite the fact that the mean sample size per locus is larger for each of the Washington populations.
- The reduction in genetic diversity within the Washington populations may be a function of genetic drift, which in turn may be the result of relatively smaller effective population sizes in Washington compared with that in Oregon and California.

#### *Genetic Differentiation*

- There is significant differentiation between each of the Washington populations, and the Oregon and California populations.
- These data support the hypothesis that each of the Washington Western Gray Squirrel populations are genetically distinct from each other, and are now functioning as separate and isolated populations.
- What these analyses demonstrate is that there is considerably more genetic differentiation between Washington and Oregon or California, than there is between Oregon and California populations.

#### *Mitochondrial Control Region Sequence Analyses*

A subset (67) of the same samples from 128 western gray squirrels used in the Microsatellite DNA analyses were used for an additional mitochondrial control region sequence analyses. Mitochondria are structures in the cell, but outside of the nucleus, which contain DNA inherited only from the mother. A 367 basepair portion of the DNA from the control region of mitochondria was sequenced (Warheit 2003).

- The haplotype is the set, made up of one allele of each gene. Haplotypes comprise the genotype (or genetic constitution of an individual or taxon).

They identified only three haplotypes from 40 Washington individuals, compared with 14 haplotypes from 27 Oregon and California individuals, and no haplotype was shared across the Columbia River.

- Genetic differences between populations can also be measured using nucleotide diversity (*i.e.*, average sequence difference). The nucleotide diversity between populations equated to long time intervals since these the Washington and California or Oregon populations diverged (roughly 12,000 to 126,000 years ago).

- Some haplotypes in Washington are more closely related to haplotypes in Oregon than other haplotypes in Washington.

Warheit (2003) summarized the results of these analyses by noting:

this study still requires additional analyses for at least three reasons. First, samples sizes need to be increased for each of the populations south of the Columbia River. Although I do not anticipate that an increase in sample size for each of the Oregon and California will significantly alter the conclusions drawn from the current data set, a greater likelihood and confidence in these conclusions will arise from more samples from Oregon and California. Second, the overall levels of genetic diversity for each of the seven microsatellite markers used in this study are low, and a greater number of microsatellite loci will provide us with a broader survey of the squirrel genome. [T]hird, we need to obtain the control region sequences for the new samples included in the expanded analysis of microsatellites. A more complete set of analyses is needed on the control region data to help understand the historical events that may have produced the phylogeographic patterns drawn from the data (*e.g.*, nested clade analysis).

Despite the preliminary nature of these analyses, the following set of conclusions have been strengthened by the inclusion of a larger sample size from the Fort Lewis and Okanogan Western Gray Squirrel populations:

1. Washington populations of Western gray Squirrels show reduced genetic diversity at both nuclear (microsatellite) and mitochondrial (control region sequences) markers compared with populations from Oregon and California. This reduction in genetic diversity may be the result of genetic drift and relatively smaller effective populations sizes.
  2. There is significant genetic differentiation between Washington Western Gray Squirrels, and squirrels from populations south of the Columbia River. Both the microsatellite and sequence data support the hypothesis that the Washington squirrels are a population(s) distinct from those in Oregon and California.
  3. There is significant genetic differentiation among the three Washington populations. \* \* \*
- Additional and more variable microsatellites should be included in any