

We did note in our emergency rule that Dr. Shaffer and his colleagues believe the divergence of Sonoma County California tiger salamanders justifies separate species recognition (Shaffer *et al.* 1993). Our DPS policy (61 FR 4722), however, does not require that levels of differentiation warranting taxonomic revision be identified for the DPS criteria to be met. In fact, our DPS policy is used for identifying groups *within* species or subspecies that may warrant listing under the ESA. Therefore, our DPS determination for Sonoma County California tiger salamander is not based on whether the divergence observed warrants separate taxonomic recognition, but rather on the relatively high divergence of the Sonoma County population from other populations of California tiger salamanders.

The Sonoma County population of California tiger salamanders is the most divergent of any population of the species. This finding is supported by the original mitochondrial DNA (mtDNA) work of Shaffer *et al.* (1993) and by additional sampling and mtDNA work of Shaffer and Trenham (2002). The “first, deepest and most significant phylogenetic split within California tiger salamander samples is between Sonoma County and all others” (H.B. Shaffer, *in litt.*, 2002). This is illustrated by the phylogenetic tree based on mtDNA in which Sonoma County California tiger salamander is the first branch after the outgroup (Shaffer and Trenham 2002). This branch is strongly supported statistically (with bootstrap probability of 100 percent) on a phylogenetic tree constructed by the neighbor joining (NJ) method. Seventy percent is the normal criterion for statistical significance of bootstrap proportions in the systematic literature (Hillis and Bull 1993 as cited in Shaffer and McKnight 1996). In addition, the branch pattern indicating that the Sonoma County population is distinct is supported by maximum likelihood and parsimony (Shaffer and Trenham 2002), two other methods of constructing phylogenetic trees (Avice 1994, Weir 1996). In addition, Shaffer and Trenham (2002) report preliminary results of analyses of two nuclear genes. These preliminary results also show that Sonoma County California tiger salamanders are genetically distinct from other California tiger salamanders. Therefore, we believe that the levels of divergence observed in Sonoma County California tiger salamanders provide substantial evidence of the significance of the population.

Shaffer and McKnight (1996) do not mention whether the divergence of

Sonoma County California tiger salamanders justifies separate species recognition. They make no statements about the taxonomic status of Sonoma County California tiger salamanders in their paper. In the Discreteness section of the emergency rule, we incorrectly attributed the statement that Sonoma County California tiger salamanders warrant separate taxonomic recognition to the 1996 publication. In fact, no Sonoma County California tiger salamanders were sampled in the study (Appendix 1 of Shaffer and McKnight 1996). The paper examined evolutionary relationships among tiger salamander species and subspecies and did not include a formal taxonomic treatment of the tiger salamander complex.

Therefore, it is not surprising that the authors did not specifically note whether or not the divergence of Sonoma County California tiger salamanders justifies species status.

*Issue 2:* One commenter also implied that the discreteness criterion of our DPS policy was only met by genetic data.

*Response:* Two professional biologists who commented reported that the Sonoma County population is geographically isolated from other populations of the California tiger salamander. We note that the proposed rule discussed the geographic separation of the Sonoma County population from other populations of California tiger salamander. The Sonoma County population is separated from other California tiger salamander populations by the Coast Range, Napa River, and the Carquinez Straits, a distance of about 72 km (45 mi).

*Issue 3:* Some commenters felt that 2 percent divergence of the Sonoma population of the California tiger salamander from the remainder of the California tiger salamander population is not meaningful or worthy of recognition as a DPS.

*Response:* We note that species boundaries are typically identified by systematists using phylogenetic analysis rather than absolute levels of sequence divergence. The intraspecific sequence divergence value of 2 percent depends on the total number of nucleotides sequenced for each gene region. This can differ significantly from species to species or from study to study and is therefore a relative value (Avice 1994, Weir 1996, Hedrick 2000). Comparisons need to be made from the same baseline. From a DPS perspective, percent sequence divergence is less important than the phylogenetic relationships depicted with strong statistical support in the neighbor joining (NJ) tree that indicate the distinctive genetic character

of Sonoma County California tiger salamanders. The phylogenetic tree (which indicates relationships among populations or groups) constructed from the mtDNA data of Shaffer and Trenham (2002) indicates that Sonoma County California tiger salamanders are very distinct relative to other California tiger salamanders, and separated from them on a branch that is strongly supported statistically. Therefore, the most comprehensive available genetic data (Shaffer and Trenham 2002) for California tiger salamanders strongly indicate that Sonoma County California tiger salamanders are distinct from other populations of the species.

*Issue 4:* One commenter analyzed Shaffer and McKnight's (1996) divergence values for various *Ambystoma tigrinum* subspecies and for California tiger salamander, finding that divergence between populations in different groups (e.g., between California tiger salamander and *A. tigrinum* subspecies and other such combinations among species and subspecies versus within a species or subspecies) had a mean of 6.37 percent and a range of 5.08 percent to 7.41 percent. The commenter states that these statistics show the 2 percent divergence of the Sonoma County California tiger salamander population is unremarkable.

*Response:* As noted above, one way to meet the significance criterion of our DPS policy (61 FR 4722) is for a population to “differ markedly from other populations of the species in its genetic characteristics” (emphasis added). We note again that our DPS policy focuses on differentiation within species (i.e., the population “differs markedly from other populations of the species”). The commenter's analysis is of differences among taxonomic groups of tiger salamanders, not of within-species or subspecies differences, which are the focus of the DPS policy.

As explained above, we believe the available mtDNA data (Shaffer *et al.* 1993, Shaffer and Trenham 2002) show that the Sonoma County population of California tiger salamander is markedly genetically divergent from other populations of the California tiger salamander. That the sequence divergence value is 2 percent is less important than the configuration of the phylogenetic tree, which strongly supports the distinctness of Sonoma County California tiger salamanders.

*Issue 5:* One commenter stated that one would expect broadly similar conclusions from allozyme and mtDNA studies and that for Sonoma County California tiger salamanders this was not the case. The commenter noted that