California Tiger Salamander Landscape Genetics Preliminary Investigation

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Table of Contents

Executive Summary **3**
Background 3
Scope of This Preliminary Investigation 4
Summary of Findings 5
Related Research and Resources 5
Major Findings 5
Gaps in Findings 6
Next Steps 7
Detailed Findings **7**
Introduction **7**
California Tiger Salamander and Hybrid Salamander Life History 8
Road Attributes and California Tiger Salamanders 10
Differences between CTS and BTS/CTS Hybrids 12
Landscape Genetics/Genomics and Conservation Genetics/Genomics 13

Means for Using Roadways and Adjacent Habitatfor Limiting the Spread of Introgression of CTS Identified During this Study 15
Creating Ponds with Short Hydroperiods 15
Creating Climbing Barriers 16
Reducing burrowing rodent populations, soil cracks, and other dry season refuges for metamorphs, juveniles and adults 16

Consultations With Researchers 16
H. Bradley Shaffer 17
Samuel Sweet 18
Christopher Searcy 20

Literature, Related Research, and Resources 20
CTS Related Genetics Papers & Presentations 25
Web Resources 27

Appendices 28
Appendix 1 Glossary 28
Appendix 2 Email Sent to Potential Interviewees 29
Appendix 3 Literature Search Methods 30

Acknowledgements 31

Executive Summary

Background

The California Tiger Salamander – *Ambystoma californiense* (CTS) is an ambystomid salamander that is endemic to California. The US Fish and Wildlife Service (USFWS) divides this species into three distinct population segments (DPS): The Sonoma County DPS, the Santa Barbara County DPS, and the Central California DPS. The Sonoma County DPS and Santa Barbara County DPS are federally listed as Endangered, while the Central California DPS is federally listed as Threatened. CTS is listed as Threatened under the California Endangered Species Act. The Cailfornia Department of Transportation (Caltrans) is required to consult with regulatory agencies about the impacts of transportation projects on CTS during state and federal Endangered Species Act (ESA) consultations as well as during California Environmental Quality Act (CEQA) and National Environmental Policy Act (NEPA) review. Because CTS is listed as either endangered or threatened under both the state and federal regulations any Caltrans action supporting species conservation and recovery should be done in coordination and close cooperation with the USFWS and the California Department of Fish and Wildlife. Caltrans also has a responsibility as a federal lead agency on behalf of the Federal Highway Administration under NEPA Assignment to support species and ecosystem conservation strategies under section 7(a)(1) of the Federal Endangered Species Act.

The conservation status of the CTS has engendered a collaborative recovery effort which includes a science advisory committee and a state wide CTS working group. Caltrans is an integral part of this recovery effort. More information about CTS biology and recovery can be found in the Recovery Plan for the Santa Rosa Plain (USFWS 2016), the Draft Recovery Plan for the Central California Distinct Population Segment of the California Tiger Salamander (USFWS 2015a), and the Draft Recovery Plan for the Santa Barbara County Distinct Population Segment of the California Tiger Salamander (USFWS 2015b). This Preliminary Investigation (PI) focuses on only one aspect of CTS recovery: that of hybridization.

A major conservation issue for CTS is the ongoing hybridization between it and the introduced non-native barred tiger salamander - *Ambystoma mavortium* (BTS). This hybridization threatens CTS with genetic extinction. Thus, CTS recovery, in part, depends on preventing the geographical spread of introgression. BTS was introduced into San Benito and adjacent counties in the central coast region, as well as other areas in California, in the 1950's and 1960's to serve as fishing bait. The two species hybridized in the areas where they came into contact creating admixed populations around the introduction sites and generating hybrid swarms. Understanding how human modifications and other landscape features like highway, rail, or transit corridors enhance or stop the movements of these salamanders is critical to prevent further introgression and to support CTS recovery actions and decision making.

For Caltrans the issue is to help prevent the spread of introgression by using roadways as barriers to dispersal and gene flow. To properly manage salamander movement in relation to highways by facilitating the movement of CTS while impeding the movement of hybrids, Caltrans needs to better understand how these animals move across the landscape (i.e. what features promote and what features impede vagility). Additionally, because Caltrans may enhance or restore seasonal wetland habitat as mitigation for CTS for projects as a result of regulatory permit conditions, it is important to understand what attributes of mitigation sites will favor development and recovery of CTS populations while impeding the development of hybrid salamanders.

Scope of This Preliminary Investigation

This Preliminary Investigation (PI) focuses on how CTS and BTS/CTS hybrids survive and move in landscapes relative to roads. The PI will identify gaps in the knowledge base and help identify practices that can be utilized now, and potential research that would be useful to Caltrans. The focus for this research is to enhance the survival and vagility of native CTS in landscapes that include roads while impeding the survival and vagility of BTS/CTS hybrids. Actions on roadways and in highway right-of-ways inhibiting the movement of hybrid BTS/CTS may become useful in the recovery of CTS and help to stop the spread of introgression.

Questions considered in this PI include:

- How do landscape attributes influence the genetic composition of CTS populations and BTS/CTS hybrid populations? Are there differences between the survival and movement of the hybrids across the landscape and the survival and movement of CTS across the landscape? What landscape attributes promote the survival and reproduction of hybrid salamander populations, and what landscape attributes promote the survival and reproduction of CTS populations? What are the gaps in the existing knowledge base?

- How much analysis of CTS and hybrid survival and movement has been done using the landscape genetics approach? Who has done this work? What methods were used for the studies (e.g. microsatellite DNA, custom-built gene capture arrays)? What are the gaps in the existing knowledge base?

- How do the attributes of roads enhance or impede the movement of CTS and BTS/CTS hybrids? What attributes of roads act as barriers to CTS and BTS/CTS hybrid movement? Attributes of roads to be considered include: right-of-way width, right-of-way landscaping, type of pavement, width of pavement, type of median, traffic density, variability in traffic density, and etc. Can existing or potential roadways be used as barriers to help isolate hybrid BTS/CTS populations and reduce or prevent further introgression? What sort of additional studies are needed to show how the attributes of roads affect these salamander populations?
- What are appropriate means for promoting the conservation of CTS and reducing or limiting the movement or survival of BTS/CTS hybrids (e.g. pool hydroperiods, right-of-way landscape vegetation, type and location of salamander crossings, gutters, curbs, fencing, and etc.? What are the gaps in the existing knowledge base?

The following areas are outside of the scope of this PI:

- Because there is already a good understanding of the locations of hybrid populations, this PI is not concerned with identifying those locations in detail.

- Similarly, this PI does not detail BTS introduction history.

- The PI also does not deal with the ecological equivalency of CTS and hybrid BTS/CTS larvae in pond/vernal pool habitat as is discussed in Searcy, Rollins, and Shaffer's 2016 paper “Ecological Equivalency as a Tool for Endangered Species Management” (Ecological Applications 26 (1): 94-103).

- Because Caltrans is not the agency responsible for determining the legal status of individual salamander populations this PI does not cover that issue. Determining the proportion of admixture that is required for an individual or a population to be considered to be protected under the endangered species acts is outside of Caltrans jurisdiction.

- This PI focuses on the use of roadways and associated features to inhibit the vagility of hybrid BTS/CTS and associated landscape genomics measures for understanding CTS and hybrid movement related to roadways. Other conservation actions are not considered in this study.

Summary of Findings

Related Research and Resources

A literature search and subject matter expert interviews discovered that a great deal of past and current research exists on the life history and genetics of CTS as well as the genetics of the hybridization with BTS. This research is summarized below in the Detailed Findings Section. Literature sources are listed below in the Literature, Related Research, and Resources section. On the other hand, CTS and BTS/CTS hybrid research related to roadways appears to be limited. The major foci of the previous work are road related mortality and tunnels for road crossings to reduce the mortality of CTS during breeding migrations (e.g. Bain 2014). Caltrans also has ongoing research on amphibian and reptile road crossings that is collecting data to develop best design practices for reptile and amphibian species that are more at-risk for encountering roads given their life history, occurrences, and distribution in California.

Major Findings

The main findings related to using the highway system as a barrier to the spread of introgression are:

- It may be possible to use the road system as a barrier to reduce or prevent the movement of BTS/CTS hybrids and limit the spread of introgression. However there are several challenges that must be overcome to practicably achieve this goal. For example it is not known if roads can be made to barriers to BTS/CTS movement while promoting the movement of other sensitive taxa.

- CTS, BTS, and BTS/CTS hybrids have limited abilities to climb over vertical obstacles. Field studies using drift fence and trap arrays were able to successfully block salamander movement using drift fences 35-60 cm tall. This trapping methodology depends on the drift fences being a barrier to salamander movement. Fences, walls, vertical climbs to culverts inlets and outlets, and etc. could be useful as barriers to hybrid BTS/CTS. However, such barriers for salamanders may also be barriers for other species.

- There will be an unknown but potentially significant maintenance requirement for maintaining roads as salamander movement barriers. Breaks in fencing and vegetation growth (which provides climbing routes) will need to be checked and fixed prior to the salamander breeding season.

The main findings related to landscape genetics/genomics are:

- While, new genomic approaches and advanced sequencing technologies provide greater opportunities for landscape genomic approaches, the principles of landscape genetics and landscape genomics are basically identical. By combining genetic analysis with geographical analysis it is possible to infer parts of the landscape that are used by animals along with migration estimates and connectivity among populations.

- Custom built gene arrays such as Single Nucleotide Polymorphism (SNP) chips are becoming commonly used in population genetic studies. Using this technology requires a major investment in equipment and time.

- Balkenhol et al. (2015) outline a three tiered conceptual framework for landscape genetics research. First, measure and understand the genetic variation in the taxa being studied. This step relies on population genetic analysis and describes the genetic composition of individuals or populations across space. Second, quantify landscape heterogeneity to understand the configuration and matrix quality of the landscape. Third, statistically link genetic variation with landscape heterogeneity and quantitatively test for landscape/genetic relationships. Each landscape genetic study is unique and care needs to be taken in the design and execution of each of the three broad tiers.

- Current work is being done with CTS to score thousands of genetic markers to be able to better track the movement of non-native alleles through the CTS population. It will be necessary to determine which genetic markers are the best ones to use in CTS and hybrid landscape genetics/genomics studies.

Gaps in Findings

- Little work has been done on how the attributes of roadways affect the movement of CTS and BTS/CTS hybrids.

- It is not known if roads can be used as barriers to hybrid BTS/CTS movement while maintaining connectivity for other sensitive taxa.

- Landscape genetics and genomics studies on CTS and BTS/CTS hybrids appear to be limited primarily to migration studies of breeding CTS and a study that determined that grassland was more of a travel barrier than chaparral.

- While experimental work shows that maintaining breeding pools with short hydroperiods, around 90-days, provides selective advantage for individuals with some CTS alleles there appears to be no field experience with controlling hydroperiods in breeding pools as a means for slowing introgression.

- There might be a potential for creating barriers to hybrid salamander movement, or at least reducing hybrid salamander movement by reducing the number of rodent burrows in highway rights-of-way, however this has not been shown by experimentation. Also some methods of rodent control could be toxic to salamanders.

- Knowledge of the movement activity of CTS is based on studies done for few populations. Many populations have not been studied in sufficient detail to understand their movement activity.

- Currently, genetic investigations on CTS and hybrids require the invasive procedure of taking tissue samples for study. There is no less invasive environmental DNA (eDNA) procedure that can be used to determine the genetic background of individual salamanders.

- It is not known if CTS and BTS/CTS hybrids move through or use upland habitat differently.

Next Steps

Caltrans could take steps to initiate or enhance research to determine how attributes of roadways affect the movement of CTS and BTS/CTS hybrids. Current Caltrans research on amphibian and reptile road crossings could be leveraged to provide a beginning for this work.

Caltrans could work with the U.S. Fish and Wildlife Service and the California Department of Fish and Wildlife to determine what locations along the state highway system are most critical for developing CTS crossing facilities or conversely to use the highway system to provide barriers to the spread of introgression. According to the CTS experts interviewed for this PI, key areas for consideration are the region along SR 246 just east of Lompoc and portions of the old Fort Ord.

Caltrans could work with the U.S. Fish and Wildlife Service, the California Department of Fish and Wildlife, the University of California, and others to develop a long-term cooperative research plan for CTS and the introgression problem. Such research should explore the landscape genomics of CTS and hybrids; using pools with short hydroperiods; reducing or eliminating rodent burrows from rights-of-way and medians; developing less invasive eDNA procedures for monitoring the success of movement barriers; and possible means (such as different substrate configurations) that will prohibit hybrid BTS/CTS passage while maintaining connectivity for other taxa.

Detailed Findings

Introduction

CTS is part of the of the tiger salamander complex. It appears to be a sister group to the other members of the complex (Shaffer et al. 2004). Historically CTS occupied a range that was both geographically and ecologically restricted. The species occurred in grasslands and open canopy oak savannahs in the Central Valley, nearby valleys (such as the Salinas Valley), and adjacent foothills from Sutter, Sacramento and Solano Counties southward to Tulare and San Luis Obispo Counties. Two disjunct population clusters occurred outside of the main range; one in the Santa Rosa area; and one in Santa Barbara County (Shaffer et al. 2004, Shaffer and Trenham 2005). Unlike BTS, CTS has an obligate biphasic life cycle with breeding and larval development taking place in pools while adults generally live in rodent burrows in the uplands making migrations back and forth to ponds to engage in breeding (Shaffer et al. 2004, Shaffer and Trenham 2005). Being dependent on vernal pools for reproduction, many populations were extirpated by the conversion of the Central Valley to agriculture.

CTS is listed under both the federal and state Endangered Species Acts. The US Fish and Wildlife Service (USFWS) divides this species into three DPS's: The Sonoma County DPS; the Santa Barbara County DPS; and the Central California DPS. The Sonoma County DPS and Santa Barbara County DPS are federally listed as Endangered, while the Central California DPS is federally listed as Threatened. CTS is listed as Threatened under the California Endangered Species Act.

In the 1950's and the 1960's BTS was introduced into central California as fishing bait primarily for introduced bass. After introduction CTS and BTS began to hybridize. Crosses between CTS and BTS produce viable fertile offspring that can breed with either parental species or with other hybrid BTS/CTS, thereby generating the conditions in which a hybrid swarm can form. In a hybrid swarm all individuals are hybrids (Allendorf et al. 2001). Presently something around 25% of the tiger salamander range in California is a hybrid swarm while another 25% of the range has been invaded by "superinvasive" chromosomal segments. Hybrid BTS/CTS may have greater fitness than native CTS in the current environmental conditions (Shaffer et al. 2015). Such hybridization leading to introgression can lead to species extinction by replacing pure populations with admixed populations that have different genetic, morphological, physiological, and ecological characteristics (Allendorf et al. 2001). Because CTS hybridization began recently and is geographically circumscribed, rapid conservation efforts to isolate the hybrids may be helpful for maintaining the species. However if such conservation efforts do not begin soon CTS may become extinct (see Allendorf et al. 2001 for a discussion of extinction by hybridization). A succinct review of the hybridization of CTS and BTS and the research literature on the topic is included in Wayne and Shaffer (2016). They also provide recommendations for conserving CTS with its complex situation.

CTS is not the only taxon for which hybridization with BTS is a problem. Populations of the Sonora tiger salamander (*Ambystoma mavortium stebbinsi*) that lives in southern Arizona and extreme northern Sonora, Mexico are also undergoing introgression with BTS (Hossack et al. 2016). These researchers suggest developing an eDNA method as a reliable minimally invasive way to distinguish between native and admixed populations. Currently genetic studies are performed on CTS by removing tissue from an animal. An eDNA approach that samples water in breeding ponds and extracts and identifies DNA from those samples could be a useful tool in monitoring the success of using roadways to reduce the vagility of hybrid BTS/CTS salamanders.

California Tiger Salamander and Hybrid Salamander Life History

The life history of CTS is detailed in a number of works including the Final or Draft Recovery Plans for the three DPS's, Shaffer and Trenham (2005), Trenham et al. (2000), Twitty (1941) and others. The life history information below is limited to that which pertains to the scope of this preliminary investigation. A major key to providing barriers to limit the spread of introgression is to understand how and when these salamanders live and move in a landscape.

CTS and BTS/CTS hybrids spend most of their lives in underground refuges, usually mammal burrows in upland areas, but they breed and undergo larval development in fishless pools and ponds. The adults must migrate between these two habitats and the metamorphs must leave ponds and travel to acceptable upland habitat, thus requiring migratory pathways between breeding and upland habitats to maintain populations (Shaffer and Trenham 2005, Trenham & Cook 2008). The life trajectory may be divided into the following stages: eggs, larvae, metamorphs (from metamorphosis through the first summer), juveniles (after the first summer until sexually mature), adults (sexually mature) (Trenham and Searcy May 2016).

Breeding activity differs by region and by year and is correlated with rainfall (e.g. Cook et al. 2010). Movement appears to be stimulated by rains that fill breeding ponds. During years with poor conditions some females may not migrate to breeding ponds (Loredo and Van Vuren 1996). While metamorphs, juveniles, and adults may move about on the surface over a period of time, mass migrations are limited to a relatively few rainy nights (Trenham and Searcy May 2016). Loredo and Van Vuren 1996 noted that the CTS in their Concord Naval Weapons Station study area typically moved to breeding ponds in waves or pulses on rainy nights or nights proceeded by rainfall. Orloff (2011) noted that adult CTS began moving to ponds with the “first substantial rain of the season” which she defined as ≥ 1 cm. The earliest capture date at her Contra Costa County study site ranged from 20 October to 11 November depending on the individual year.

The typical breeding habitat for CTS is fishless seasonal or semi-permanent ponds (Shaffer and Trenham 2005). Eggs hatch 2 to 4 weeks after deposition (Barry & Shaffer 1994). Prior to the European period CTS probably were confined to breeding in shallow and relatively temporary vernal pools. Thus, these salamanders evolved to have a brief larval period metamorphosing in a short time. With the introduction of pastoralism, urbanization, and intensive agriculture the hydrology in much of CTS’s range was greatly modified along with the vegetation of the California grasslands (Simms 1988, Johnson et al. 2013). Many vernal pools were lost and many cattle and agricultural ponds with much longer hydroperiods and greater depths were built (Johnson et al. 2013). With the construction of cattle ponds the salamanders began to use these ponds as breeding habitat (cf. Alvarez 2004). When BTS was introduced into California the introduced salamanders were often (but not always) released into habitat already occupied by CTS and the two species began to interbreed. The hybrids appear to be fully fertile and capable of thriving in the current habitat conditions and hybrid swarms formed as the introgression spread.

Males tend to arrive at breeding ponds and stay at breeding ponds longer than females (Loredo and Van Vuren 1996). Adults leave breeding ponds at night soon after egg deposition. Loredo et al. (1996) using visual tracking over one night noted a maximum travel distance of 130 m. Trenham (2001) using radio tracking detected a maximum travel distance of 248 m. However these studies only considered the initial movement from ponds into terrestrial habitat. Trenham and Shaffer (2005) reported trapping 50% of adults with a trap line 150 m from a breeding pond and 95% of adults at 620 m from a breeding pond. A few individuals moved up to 1000 m from the most probable breeding pond. CTS have been reported to have been found up to 2.2 km from breeding ponds (USFWS 2015a citing Orloff, S. G. 2007. Migratory movements of California tiger salamander in upland habitat – A five-year study, Pittsburg, California. Prepared for Bailey Estates LLC.). Orloff (2011) detected large numbers of CTS farther from breeding ponds than were detected in previous studies. She also detected some upland habitat site fidelity. Being underground for the majority of the time makes direct observation and study of adult salamanders difficult.

The breeding and development period is governed by the amount and pattern of rainfall in relation to the volume of water that a pool can hold. The length of the inundation period may influence when the peak of metamorphosis occurs with the peak of metamorphosis occurring later in wetter years when inundation periods are longer (Loredo and Van Vuren 1996). Loredo and Van Vuren found in their study that larger CTS larvae metamorphosed early and that smaller larvae continued to grow until metamorphosis. They speculated that large larvae may be selected to metamorphose early. The disadvantage to later metamorphosis is the danger of the pond drying.

Trenham and Searcy (May 2016) indicate that the average date of metamorph emergence is as early as 12 May and as late as 19 June with the average number of days from breeding to emergence ranging from 88 days to 178 days. Metamorphs can emerge as late as August (Searcy and Shaffer 2016 based on Trenham et al. 2000). At Jepson Prairie between 2004-2005 and 2012-2013 inclusive the metamorph emergence period started as early as 7 May and as late as 2 June with an overall start date of 17 May. During the same period the metamorph emergence period ended as early as 18 May and as late as 10 July with an overall end date of 3 July. The metamorph emergence period ends as the pools dry out (Trenham and Searcy May 2016). At Loredo and Van Vuren's (1996) study site metamorph movement away from the pond could not be linked to any of the environmental variables measured. However, at one point a "disproportionate number" emigrated during a rainstorm. At Concord emigration of metamorphs occurred from June 3 through July 27 in 1992 and June 1 through August 22 in 1993. Several metamorphs were found near Stanford University in crevices in the sandbag embankment between Lake Lagunita and Junipero Serra Boulevard during 1992 on unreported dates after February (Barry and Shaffer 1994). Rarely, metamorphs may exhibit mass movements in the summer (Holland et al. 1990). Metamorph production varies greatly among years. Dry conditions prevented a significant numbers of salamanders from metamorphosing in 1994 at the Concord study site (Loredo and Van Vuren 1996).

Road Attributes and California Tiger Salamanders

It is well known that the attributes of roads can influence the connectivity of wildlife populations. Usually road ecologists are interested in promoting connectivity across roadways and other linear infrastructure corridors and having these linear corridors be more permeable to wildlife. In the case of hybrid BTS/CTS the normal situation is turned on its head and the interest is in preventing the hybrids from moving across the roadway.

Roads are known to impede or completely block the movement of amphibians across a landscape (Langen et al. 2015, van der Ree et al. 2015b). The attributes of roads that may impact an organism extend beyond the travelled way and include noise, light, chemical pollution, disturbance effects, and habitat modification. The area over which a road affects organisms is the road effects zone (van der Ree et al. 2015). The environmental impacts in a road effects zone are usually greatest near the roadway and diminish with increasing distance from the roadway. According to van der Ree et al. (2015) the size of the road effects zone is influenced by the physical attributes of the road, the volume and nature of the traffic, the adjacent landscape, climate, and individual species traits.

The road effects zone is a useful concept in examining the impact of roadways on the vagility of CTS and BTS/CTS hybrids because it ecologically places the roadway itself within the landscape. However very little research exists for this subject. There is a considerable amount of general information and speculation about the impacts of traffic noise, vibration, and light, (Langen et al. 2015). But there is little if anything specifically about CTS and hybrids.

CTS are known to move over roads during dispersal or migration which can result in mortality due to passing vehicles (Twitty 1941, Bain 2014, USFWS 2014, USFWS 2015a, and USFWS 2015b). Thus, at least in some circumstances the salamanders do not avoid roads. Large numbers of salamanders are usually found on roadways only during mass migrations (Barry and Shaffer 1994). Twitty recorded mass migrations across Mayfield Road near Stanford University on the rainy nights of January 1 and January 3, 1940 (Twitty 1940, Barry and Shaffer 1994). Two road-killed adult salamanders were found near Stanford University on Junipero Serra Boulevard at Lake Lagunita in January and February of 1992 (Barry and Shaffer 1994).
Bain (2014) examined tunnels constructed to enhance CTS movement and reduce road-kill mortality along Stony Point road in Sonoma County. For the purposes of this PI Stony Point Road can be used as an example of a roadway that has attributes that do not form a complete barrier to CTS migration. During the study Stony Point Road was a two lane asphalt paved road elevated 1 to 1.5 m above the surrounding terrain. The road had steep earthen shoulders and was flanked in the study area by shallow ditches. The tunnels designed to promote salamander crossing were 22 m long which gives an indication of the road width. Two types of fencing were used to prevent CTS from getting onto the roadway and to direct them to the crossing tunnels: plastic mesh fencing and solid plastic fencing. However, not all of the upland habitat was separated from the road by fencing. The fencing results were inconclusive.

The review of the literature and the practitioner survey indicate that it is possible for roadways to be used as barriers to movement for hybrid BTS/CTS salamanders. However, additional research is warranted to identify the most cost effective means for creating barriers. Both CTS and hybrid BTS/CTS must be able to migrate between breeding ponds and upland habitat to be able to complete their life courses. If a road is between breeding ponds and suitable dry season habitat disruption of this movement will reduce or eliminate the salamander population. Creating barriers to hybrid salamander movement will also inhibit the further spread of introgression by preventing hybrid individuals from entering unhybridized CTS populations.
Among the possible means of creating barriers to hybrid BTS/CTS movement are:

- Using unclimbable fences and walls adjacent to roadways to prevent salamanders from crossing roads,
- Developing unclimbable end treatments for culverts,
- Reducing burrowing rodent populations, soil cracks, and other dry season refuges for metamorphs, juveniles and adults
- Reducing the hydroperiod of pools and ponds to select for alleles that promote the rapid metamorphosis of native CTS.
- Not building to promote salamander crossing on highways in areas with hybrid BTS/CTS.

In their study in Contra Costa County Loredo and Van Vuren (1996) used a drift fence of 50 cm high aluminum flashing with a lower edge that was buried from 10 to 15 cm in the ground. This produced a fence that was between 35 and 40 cm tall. Bain (2014) used 43 cm tall fencing in her Sonoma County study to guide CTS to crossing locations. Plastic mesh was used when water flow or wind needed accommodation and solid plastic was used in other locations. The fencing as it was designed was not effective in reducing road mortality. Possible explanations include CTS use of habitat on the road-ward side of the fence and salamanders walking around the edge of the fence. Orloff used 90 cm tall silt fence buried 30 cm in the ground to produce a 60 cm tall fence. She replaced this fence each year of her study. She also patrolled the fence and repaired it as necessary during the study. Because of the size of the study area not all of the area was fenced. Eight individual salamanders that were relocated outside of the study area were later captured within the study area. This number is less than 1% of the total captures. It is not known how long an unclimbable fence needs to be to prevent either CTS or hybrids from walking around the end of the fence and attempting a road crossing.

It must be understood that using roads as barriers for hybrid salamanders may also inhibit the vagility of other non-target organisms. Other sensitive amphibians that could be negatively affected include California red legged frog (*Rana draytonii*), western spadefoot (*Spea hammondii*) and California newt (*Taricha torosa*) (H. Bradley Shaffer pers. com.).

Fencing for amphibians is reviewed in Clevenger and Huijser (2011), Huijser et al. (2015), and Hamer et al. (2015). The information in these references will need to be adapted for CTS and hybrids. Caltrans also has ongoing research on amphibian and reptile road crossings that is dealing with amphibian fencing.

Differences between CTS and BTS/CTS Hybrids

Depending on their genetic composition hybrid BTS/CTS may or may not be phenotypically distinct from CTS. CTS larvae often tend to be smaller than hybrid larvae (Trenham and Searcy 2016). There are also paedomorphs (sexually mature larvae) in hybrid populations but not in native CTS populations (Trenham and Searcy 2016). However, Alvarez (2004) discovered overwintering (but not sexually mature) CTS larvae in permanent ponds in Contra Costa County. Larval body size over 15 cm, presence of paedomorphs, an unusual coloration pattern on larvae, and barred rather than spotted adults are indicative of hybrid BTS/CTS populations. However, genetic testing is required for certain identification of hybrid populations. Populations with "superinvasive" chromosomal segments may not show distinct phenotypic differences from native CTS populations (Trenham and Searcy 2016). Work by Johnson et al. (2010) indicated that hybrid BTS/CTS can have better locomotor performance than CTS which may promote the spread of hybridization.

Fitzpatrick & Shaffer (2004) roughly classified salamander breeding habitat in the CTS and BTS hybrid zone as: vernal pools, ephemeral cattle ponds, and perennial ponds. Vernal pools, which are the natural breeding habitat for CTS are usually natural shallow isolated depressions that fill with winter rainfall and dry out in the late spring. These pools are characterized by a distinctive vegetation and diverse invertebrate fauna. Because the pools dry out they are fishless. Pacific chorus frogs, western toads, and spadefoot toads may also breed in these pools providing prey for the carnivorous CTS larvae. Fitzpatrick & Shaffer (2004) note that vernal pools are now rare in their study area due to habitat modification. Ephemeral cattle ponds are pools that generally have a longer inundation period than natural vernal pools. The presence of the cattle greatly modifies the flora and fauna of the pool due to the input of nutrients and soil compaction. If cattle are not allowed to access the ponds over time they develop some of the characteristics of vernal pools. Perennial ponds generally maintain water throughout the year thereby enhancing the reproduction of BTS/CTS hybrids with their longer larval times and their potential for paedomorphy. In Fitzpatrick and Shaffer's study area nonnative alleles predominated in perennial ponds. There was a genetic difference between ephemeral pools and perennial ponds even if they were separated by short distances. This may be due to selection for some BTS alleles in perennial ponds Fitzpatrick & Shaffer (2004).

The hybrid BTS/CTS tend to spend a longer time as larvae and grow to larger size prior to metamorphosis than CTS. Additionally paedomorphs are known for BTS, but not for CTS. Searcy (2010) noted that larger CTS juveniles and adults had a higher survival rate than smaller ones and they also moved farther than smaller ones. BTS/CTS hybrid breeding ponds with longer hydroperiods generally produce metamorphs with greater masses than breeding ponds with shorter breeding periods.

Thus, the longer hydroperiod usually occurring in stock ponds selects for individual salamanders with BTS alleles that lead to longer larval periods. Work by Johnson et al. (2013) indicates that maintaining hydroperiods of around 90-days could be of benefit to maintaining CTS populations or CTS alleles in hybrid populations. Their suggestion and reasoning is as follows:

> Cumulatively, these results suggest that reduction in hydroperiod may be a viable conservation strategy, which is likely to be most effective at the edge of the hybrid zone. Pond drying, or any period during which the aquatic environment deteriorates beyond the physiological limitations of gilled salamanders (e.g. high temperature, low dissolved O2), should eliminate paedomorphic adults for that season. Annual cycling through such conditions should render paedomorphosis an unviable life history strategy, although individuals carrying paedomorphosis alleles would still exist in the terrestrial adult population. Alternatively, an early drying regime ~90 days subsequent to the first detection of hatchling larvae in the pond could serve to select against hybrids or at least eliminate hybrid advantage with respect to size at metamorphosis. If such a strategy was implemented along the periphery of the hybrid swarm, the potential for spread of alleles associated with hybrid advantage could be minimized. (Quoted from Johnson et al. 2013 p. 563.)

Landscape Genetics/Genomics and Conservation Genetics/Genomics

Since the middle 1990's the rapidly advancing technologies for sequencing genomes along with polymerase chain reaction (PCR) technology and bioinformatics techniques have revolutionized biology. As sequencing costs rapidly decline genetics/genomics are becoming much more important in both conservation and evolutionary biology. While population genetics theory and genetic testing have been important for many years in evolution and ecology the older techniques were both time consuming and expensive (see for instance Ford 1975). With the development of modern genetics procedures, particularly PCR, widespread application of genetics to biological conservation problems became practicable. The Journal Conservation Genetics was started in 2000 and the first textbook published in 2002 (Primmer 2009). Over this same time period rapid advances in GPS, GIS, and other spatial systems allowed finer detail in the study of organisms over different landscapes. These two sets of advances combined to generate landscape genetics/genomics (Storfer 2015). Many consider the field of landscape genetics to have been formally inaugurated by Manel et al. in 2003 (Storfer 2015).

Storfer et al. (2010) defined landscape genetics as: "research that explicitly quantifies the effects of landscape composition, configuration and [or] matrix quality on gene flow and [or] spatial variation" and additionally characterized landscape genetics as "…a rapidly growing field that integrates data and analysis methods from landscape ecology, spatial statistics, geography and population genetics to understand the spatial distribution of genetic variation." Allendorf et al. 2010 defined landscape genomics as: "The study of many markers in genes under selection, in spatially referenced samples collected across a landscape and often across selection gradients. It uses comparisons of adaptive and neutral variation to quantify the effects of landscape features and environmental variables on gene flow and spatial genetic variation".

The basic difference between Storfer et al.'s (2010) definition of landscape genetics and Allendorf et al.'s (2010) definition of landscape genomics is that Allendorf et al. make explicit mention of using many genetic markers to study the effects of the landscape. A recent definition of landscape genetics that provides a bridge between Storfer's and Allendorf's definitions is provided by Balkenhol et al. (2015). Landscape genetics is "research that combines, population genetics, landscape ecology, and spatial analytical techniques to explicitly quantify the effects of landscape composition, configuration, and matrix quality on microevolutionary processes, such as gene flow, drift, and selection, using neutral and adaptive genetic data." The information gathered can be used to help conserve an individual taxon or groups of taxa.

While, new genomic approaches and advanced sequencing technologies provide greater opportunities for landscape genomic studies the principles of landscape genetics and landscape genomics are very similar (Sunnucks and Balkenhol 2015). By combining genetic analysis with geographical analysis it is possible to infer parts of the landscape that are used by animals, migration estimates, and connectivity among populations (Shaffer 2015). McCartney-Melstad and Shaffer (2015) review key recent amphibian studies dealing with landscape genetics and genomics noting the study methodologies and conclusions. Storfer's 2015 web posting provides a basic bibliography for landscape genetics. Balkenhol et al. (2015) provide a recent comprehensive text book for the rapidly evolving field.

Balkenhol et al. (2015) outline a three tiered conceptual framework for landscape genetic research. First, measure and understand the genetic variation in the taxa being studied. This step relies on population genetic analysis and describes the genetic composition of individuals or populations across space. Second, quantify landscape heterogeneity to understand the configuration and matrix quality of the landscape. Third, statistically link genetic variation with landscape heterogeneity and quantitatively test for landscape/genetic relationships. Each landscape genetic study is unique and care needs to be taken in the design and execution of each of the three broad tiers. One caution to be considered in using a landscape genomics approach is the inherently great complexity both in the genome and in the landscape. Connectivity modeling can quickly become computationally burdensome (Leonard et al 2016). The appropriate statistical methods and software need to be used to disentangle the complexity and model the reality of the interaction of organisms and their environment.

The identification of barriers to gene flow is frequently considered in landscape genetic research (Storfer 2010, Emel and Storfer 2012, Ishiyama et al. 2015). It is possible to use assignment tests to help infer what landscape features impede or enhance vagility (Sunnucks and Balkenhol 2015). However, features such as highways may have been in place for too short a time for a genetic pattern to develop (Shaffer et al. 2015). Selectively neutral DNA can be used to understand the dispersal of organisms. Using the proper methods, impedance of animal movement by roads can be detected after very short time intervals. Thus, reduced dispersal and gene flow due to the presence of roads can be determined prior to demographic impacts (Sunnucks and Balkenhol 2015). In the case of isolating BTS/CTS hybrids and stopping spread of introgression it is possible to use landscape genetic/genomic methods to help determine which road features can be used to isolate appropriate populations. Assignment and parentage tests may be useful for rapid determination of vagility reduction in BTS/CTS hybrids (cf. Sunnucks and Balkenhol 2015). However, sufficient sample sizes and robust study designs are required. One hurdle to using a landscape genomic approach to CTS and hybrid studies is the sheer size of the very large salamander genome (Shaffer et al. 2015). Wilkinson et al. (2011) discuss a methodology for developing the most informative SNP chips for determining the ancestry of an organism.

Landscape genetics and conservation are clearly related. Genetic information about how populations actually use and move through a landscape can be vital when designing conservation areas and the corridors that allow movement among them (Shaffer et al. 2015). For BTS/CTS hybrids the goal is to develop impassible terrain to prevent the extinction of CTS by introgression with BTS. In the case of using roadways as barriers to hybrid BTS/CTS movement Caltrans needs to focus on an applied study of roads as barriers to salamanders and to measures that select for CTS alleles. This study can be linked to broader CTS conservation efforts, but study activities must be directly related to Caltrans transportation mission.

A major determinant in the cost of doing research will be the number and types of genetic markers used. Allendorf et al. (2010) note that hybridization can be detected and the proportion of admixture estimated by using 10’s of loci. However, because the introgression rates of different loci varies it is possible that using a small number of loci (i.e. around 10 neutral markers) may not be adequate to detect hybridization. Using a larger number of loci can help increase the ability to determine kin relationships among organisms. For the CTS hybridization problem understanding kin relationships will help determine if a particular experimental treatment is a barrier to hybrids. Allendorf et al. (2010) noted that a study methodology using 377 microsatellites in humans was able to resolve between closely related groups. Similar approaches should be able to help determine if hybrid BTS/CTS are able to cross over experimental barriers. However, further work is needed to determine what number and type of markers are necessary to be able to make the distinction between CTS and hybrids. Also estimating the proportion of admixture in an individual or describing the dynamics of introgression may require many markers (Allendorf et al. 2010, but see Wilkinson 2011).

There is a considerable amount of work on CTS and hybrid genomics currently being done particularly at the Shaffer Lab at UCLA. McCartney-Melstad and Shaffer (2015) report that currently “thousands of markers throughout the genome at hundreds of ponds for samples collected over the past 30 years to track the movement of non-native alleles in real time…” Caltrans could become part of this effort.

Means for Using Roadways and Adjacent Habitat for Limiting the Spread of Introgression of CTS Identified During this Study

The review of the literature and the practitioner survey indicate that it might be possible for roadways to be used as barriers to movement for hybrid BTS/CTS salamanders. However, additional research is needed to identify the most cost effective means for creating barriers. Both CTS and hybrid BTS/CTS must be able to migrate between breeding ponds and upland habitat to be able to complete their life courses. Creating barriers to hybrid salamander movement will inhibit the further spread of introgression by preventing hybrid individuals from entering unhybridized CTS populations.

Creating Ponds with Short Hydroperiods

As noted above in ‘Differences Between CTS and BTS/CTS Hybrids’, long breeding pool hydroperiods favor hybrids with genes for longer larval times leading to larger metamorphs. As a measure for conserving CTS Caltrans could partner with other agencies or conservation organizations to develop short period breeding ponds.
However, it must be noted that this activity would require careful pond design and active pond management for the lifespan of the pond. The design of the pond would have to consider a

source of water, how to hold a sufficient quantity of water for the desired larval development period, and how to remove water from the pond to maintain a short salamander larval development period of around ninety days. A pond would have to be monitored to determine when salamander eggs hatched and larvae began to develop to determine the beginning of the circa ninety day development period. Additionally, habitat for metamorphs, juveniles and adults would have to be maintained in the vicinity of the pond.

Creating Climbing Barriers

- Using unclimbable fences and walls adjacent to roadways to prevent salamanders from crossing roads,
- Developing unclimbable end treatments for culverts,

As detailed under road attributes, the literature, and discussions with experts CTS, BTS, and BTS/CTS hybrids have a limited ability to climb smooth vertical surfaces. It appears that a vertical climb around 50 cm high will be a barrier for these salamanders. Climbing barriers will have to be maintained without steps, gaps, and vegetation providing means for the salamanders to surmount the obstacle.

Reducing burrowing rodent populations, soil cracks, and other dry season refuges for metamorphs, juveniles and adults

Because salamanders require relatively moist and relatively cool habitat to prevent desiccation CTS and the hybrids both need to find appropriate upland habitat for hot dry summer conditions. The dry season is spent in rodent burrows (often California ground squirrels – *Otospermophilus beecheyi* and valley pocket gophers *Thomomys bottae*) or cracks in the soil (Loredo et al. 1996, Barry and Shaffer 1994, Shaffer and Trenham 2005). These locations provide greater protection from desiccation for the salamanders than surface features such as rocks and logs. Salamanders unable to find the appropriate fossorial habitat may succumb to the hot dry conditions experienced in CTS range (Holland et al. 1990). There was a significant mass mortality of juveniles at Grant's Lake in the summer of 1983 after an unusual summertime mass movement. The shore of the lake lacked rodent burrows and many juveniles appeared to succumb to dehydration due to being unable to find good upland habitat (Holland et al. 1990). As a conservation measure Cook et al. (2006) constructed artificial habitat for pocket gophers in Sonoma County that was quickly colonized and did provide some upland habitat for some CTS. The heavy use of rodent burrows by adult salamanders yields the potential opportunity to reduce hybrid salamander migration by reducing the number of, or eliminating, rodent burrows in highway rights-of-way and medians.

A precautionary note for rodent control was provided by Dr. Samuel Sweet. He mentioned that much rodent control is achieved by using poisoned bait. However, rodent control using poisoned bait might result in CTS mortality if the bait is carried by a rodent into a burrow. The conditions in burrows tend to be cooler and more humid than conditions outside potentially leading to condensation, with toxins leaching from the interior of the bait. Salamanders and other organisms can come into contact with the bait and absorb the toxic material through their belly skin. Dr. Sweet advises Caltrans against using poisoned bait to isolate BTS/CTS hybrids.

Consultations With Researchers

I contacted the individuals below to gather information for this investigation.

H. Bradley Shaffer

H. Bradley Shaffer is the Principal Investigator at the Shaffer Lab at UCLA. Dr. Shaffer is one of the preeminent authorities on CTS and the hybridization of CTS with BTS. He has published in numerous reviewed articles on CTS genetics and ecology.

Using roads to block the movement of hybrid California tiger salamanders (*Ambystoma californiense,* CTS)/*A. mavortium* BTS hybrids) may be a useful management strategy under some circumstances. However, using roads for this purpose can also cause collateral damage to other sensitive animal populations that occupy the same habitats and which must cross roads to migrate or maintain connectivity. Other sensitive amphibians that could be negatively affected include California red legged frog (*Rana draytonii*), western spadefoot (*Spea hammondii*) and California newt (*Taricha torosa*).

Currently, there are no known methods for using roads to block migration and dispersal of hybrid CTS/BTS while maintaining connectivity for other sensitive taxa. A conundrum may exist in that it might prove to be impossible to develop treatments that maintain connectivity for other organisms while blocking the spread of hybrid CTS/BTS. Difficult decisions about how to treat individual populations of salamanders may have to be made to develop and implement conservation strategies that can limit the spread of salamander introgression while at the same time limiting the collateral damage occurring to other sensitive species populations.

Carefully designed and executed research could help solve the problem of how to block hybrids while limiting the collateral damage to other organisms and possibly maintaining porosity of the road for other sensitive taxa. For example, experiments could determine if there are animal crossing designs (e.g. types of substrate, placement of crossing structures) that act as a barrier to the hybrid salamanders while maintaining connectivity for other species. The experiments would have to be properly designed taking into consideration the habitat conditions and the populations both of salamanders and other sensitive taxa in the locations where the road treatment would be located. BTS populations that were introduced in the vicinity of Clear Lake and in Siskiyou County are not useful surrogate populations for this research. These areas of introduction are outside of the native range of CTS and are located in habitats that do not reflect those where introgression is occurring. Additionally these areas harbor only pure BTS, but the critical need is to block movement of CTS/BTS hybrids. Extensive published research indicates that hybrids often have different, sometimes superior abilities. For example hybrids appear to be able to disperse farther than either CTS or BTS.

There are two areas where blocking the spread of introgression would have a particularly large, immediate impact for maintaining native CTS populations. These are 1) the Santa Barbara County DPS where a population of BTS have existed for many years on the grounds of the Lompoc federal prison, and 2) the old Fort Ord site in Monterey County at the leading edge of the major hybrid zone expansion of the Central California DPS. The native Santa Barbara County DPS is located in northwestern Santa Barbara County and is federally listed as endangered. There is an introduced BTS population near the Lompoc Federal Prison. This introduced population is spreading and beginning to come into contact and interbreed with the native Santa Barbara County DPS of CTS. Hybrids have been found adjacent to State Route 246 near Cebada Canyon Road, and the further spread of the hybrids might be stopped by appropriate road modifications. The old Fort Ord Military Reservation currently sits at the leading edge of the hybrid swarm movement from the Salinas Valley north, with State Route 68 serving as a potential barrier to further movement north. Additional roads within Ft Ord, currently managed by either the military or BLM, might also serve as barriers to dispersal. These animals are part of the Central California DPS which is listed as federally threatened. The introgression boundary area in the old Fort Ord is a potential area for experimental work on trying to contain the spread of introgression, and could serve as a model for such road activities throughout the Salinas and Central Valleys.

Features to block hybrid salamander movement will fail over time if maintenance is not properly done. Thus, proper maintenance is imperative. Maintenance includes repair of the original features, vegetation control, and trash removal. It also should include regular genetic monitoring of salamander populations on either side of the road to determine whether the road activities are acting as a barrier to hybrid movement.

Samuel Sweet

Dr. Samuel Sweet is a Professor in the Department of Ecology, Evolution and Marine Biology at the University of California at Santa Barbara. He is an authority on the distributional ecology and systematics of North American and Australian amphibians and reptiles including the mechanics of intergrade zones and speciation, areas in which he has published extensively. Dr. Sweet has extensive experience with CTS and BTS particularly with the Santa Barbara County DPS.

Dr. Sweet indicated both in his email and in his conversation that there does not appear to be an easy passive means of blocking the movement of BTS/CTS hybrids while allowing the passage of CTS.

The population of tiger salamanders in the Salinas Valley is thoroughly introgressed. That is not the case yet in the Santa Barbara County DPS where the majority of the local populations still consist of pure CTS. There are, however, hybrid salamanders in this population segment. BTS was released into the pond on the Federal Correctional Complex, Lompoc a number of years ago for use as fishing bait. Today the pond at the prison complex contains a pure BTS salamander population, and hybrid salamanders have spread away from this pond into the surrounding area. Thus, the pond adjacent to SR 246 near Cebada Canyon Road contains hybrid BTS salamanders. It is important to contain the movement of the hybrid salamanders or to eliminate the hybrid populations to conserve the endangered Santa Barbara distinct population segment. However it is difficult to either study or take conservation actions in the vicinity of Lompoc due to the requirements of the prison complex and to restrictions for working on private property. One confirmed F1 hybrid was handled and released at La Purisima Golf Course east of Lompoc in 2009.

A key issue for the conservation of CTS throughout its range is reducing salamander mortality while they are crossing roads or entering developed habitat during migrations between breeding ponds and suitable upland habitat. It is important for Caltrans to develop, emplace and maintain crossings that permit CTS to migrate across roadways. A possible conservation strategy to reduce mortality during breeding migrations is to use impassible barriers to divert salamanders away from habitat where they have a high mortality risk and to habitat where the mortality risk is lower. An example is a location where salamanders could be directed away from the parking lot of a business park and toward suitable upland habitat without automobiles and with rodent burrows.

Another focus of the conversation was on methods of attempting to eliminate hybrids from the effective populations of ponds while maintaining the native salamanders in those populations. Hybrids but not native CTS may breed in August and September prior to the normal fall rains in California. CTS evolved to survive in the California climate where vernal pools do not fill until rain occurs later in the year. Any salamanders breeding in long hydroperiod ponds prior to the rainy season will be hybrids. Thus, salamanders in pools during the late summer and until the start of heavy rains in fall can be removed from the effective population without affecting native CTS.

When they are migrating to ponds at the beginning of the breeding season both the CTS and hybrid salamanders follow topographic lows and they can be guided into ditches which they will follow to the breeding ponds. Migrating adults will ride the water in gutters to a pond. This tendency to follow channels can be used to artificially select for native genes in a population. Salamanders returning to ponds from upland habitat can be guided to steep-sided basins that preclude salamander escape. The salamanders in the holding pools could be surveyed and those exhibiting BTS morphological characteristics or genes could be removed from the effective population while those exhibiting CTS characteristics or genes would be included in the effective population. This would need to be done soon after the rainfall event, because females do not remain in breeding condition for more than a day or two. Metamorphs appear to radiate out from breeding ponds when they go to upland habitat and do not follow topographic lows. Thus they are much harder to protect in principle, but no less important to the health of the population.

Because BTS and BTS/CTS hybrids are adapted to deeper longer hydroperiod ponds Caltrans could consider building ponds that are shallow and have short hydroperiods which select for CTS. BTS can produce sexually mature paedomorphs but, CTS cannot and must metamorphose to reproduce. Also gilled axolotl type hybrid BTS/CTS can metamorphose. Hybrid salamanders in longer period ponds grow larger than native CTS and can become sexually mature in 2 years rather than the normal 3 or 4 years for CTS. The last larvae in remaining long period ponds will be non-native animals.

Another potential conservation strategy could be to place barriers around a pond in which the salamander population is beginning to undergo hybridization, but which has heretofore contained CTS. Hybridization proceeds quickly. If a location is discovered to be undergoing hybridization prior to the hybrids undergoing metamorphosis and occupying upland habitat it may be possible to remove the hybrids and prevent adults in the uplands from moving into the ponds until the hybrids are extirpated. Once the hybrids in the pond are removed, reoccupation of the pond by pure CTS from the uplands could be allowed. Metamorphs can disperse up to ½ mile to reach rodent burrows and adult CTS can spend several years in the upland without breeding. This ability to survive multiple years without breeding appears to be a trait that evolved to allow individuals and hence populations to survive the multi-year droughts common in CTS's range. Non-natives could be extirpated from ponds in three to four years.

It is not known whether or not adults feed in breeding ponds. It is also not known if there are differences in upland habitat selection between CTS and hybrids. Upland habitat studies of these salamanders would be difficult due to the underground habits of the salamanders.

Rodent control using poisoned bait might result in CTS mortality if the bait is carried by a rodent into a burrow. The conditions in burrows tend to be cooler and more humid than conditions outside potentially leading to condensation, with toxins leaching from the interior of the bait. Salamanders can come into contact with the bait and absorb the toxic material through their belly skin.

Dr. Sweet recommended contacting Dr. Bradley Shaffer.

Christopher Searcy

Dr. Christopher Searcy is an Assistant Professor at the University of Miami, Department of Biology. Dr. Searcy has extensively studied CTS and published several papers on CTS related subjects including: calculation of mitigation credits; ecological equivalency of larval CTS, BTS/CTS hybrids and BTS; microhabitat use, and migration distance. He is the senior author of papers reporting studies on habitat use and migration distance of CTS conducted at Jepson Prairie near Davis, CA.

At Jepson Prairie the researchers installed a series of 165 drift fences with associated pitfall traps near Olcott Lake and Round Pond. Data from the trapping effort was analyzed and the maximum ecophysiological migration distance was estimated to be about 2484 m a year although most salamanders move considerably less distance away from breeding ponds. One recaptured individual travelled nearly 2 km. The maximum migration distance for CTS is one of the longest for salamanders and the ability to travel long distances overland helps spread BTS genes through the CTS population via hybridization. Little appears to be known about the differences in vagility between CTS and BTS/CTS hybrids.

Migrating CTS are poor climbers, a trait that can be used to create barriers to movement. The CTS studies at Jepson Prairie were unable to surmount vertical barriers of 1 ft. (.3048 m). The salamanders were effectively trapped in pitfall traps 1 ft. deep. Roads flanked by fences or walls or having solid median barriers could prevent migration of hybrid salamanders. Proper maintenance would be needed to insure the integrity of the barriers.

Dr. Searcy suggested contacting H. Bradley Shaffer and David Cook who have worked with CTS.

Literature, Related Research, and Resources

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Both metamorphs and adults were tracked using a drift fence array. The data indicated that for both juveniles and adults survival in the uplands during the dry season was higher for animals with greater masses. Animals with greater masses also dispersed farther than did animals with smaller masses.

Searcy, Christopher A. and H. Bradley Shaffer. 2016. Do ecological niche models accurately identify climatic determinants of species ranges? The American Naturalist 187 (4): 423-435.

Searcy, Christopher A., Hilary B. Rollins, and H. Bradley Shaffer. 2016. Ecological equivalency as a tool for endangered species management. Ecological Applications 26 (1): 94-103.

Shaffer, H. Bradley and Peter C. Trenham. 2005. *Ambystoma californiense* Gray, 1853 California Tiger Salamander. *In* Lannoo, M. (ed.), 2005. Amphibian Declines: The Conservation Status of United States Species. University of California Press, Berkeley, CA.
This species article provides a brief synopsis of the basic life history, distribution, and conservation information of CTS known at the time of writing. It includes a little information on the hybridization between CTS and BTS. This account is available at the AmphibiaWeb website: http://amphibiaweb.org/cgi/amphib_query?where-genus=Ambystoma&where-species=californiense

Shaffer, H. Bradley; Müge Gidiş, Evan McCartney-Melstad, Kevin M. Neal, Hilton M. Oyamaguchi, Marisa Tellez, and Erin Toffelmier. 2015. Conservation genetics and genomics of amphibians and reptiles. Annual Reviews of Animal Biosciences 3: 113-138.

Sims P. L. 1988. Grasslands p. 265 to 286. In Barbour, M.G. and W. D. Billings (eds.) North American terrestrial vegetation. Cambridge University Press, Cambridge, UK. 434 pp.
In his section on California grasslands Sims briefly summarizes the virtual replacement of native perennial bunchgrasses with introduced annual grasses along with the shift in grazing patterns due to the introduction of cattle.

Storfer, Andrew; Melanie A. Murphy, Stephen F. Spear, Rolf Holderegger, and Lisette P. Waits. 2010. Landscape genetics: where are we now? Molecular Ecology 19: 3496–3514.

Sunnucks, Paul and Niko Balkenhol. 2015. Incorporating Landscape genetics into Road ecology. P. 110 to 118. *In* Rodney Van Der Ree, Daniel J. Smith, and Clara Grilo (Eds.). Handbook of Road Ecology. Wiley Blackwell, Chichester, UK.
This chapter provides a brief general overview of how to use landscape genetics to determine the relationship of biota to roads.

Trenham, Peter C and David G. Cook. 2008. Distribution of migrating adults related to the location of remnant grassland around an urban California tiger salamander (*Ambystoma californiense*) breeding pool, Chapter 2. In Joseph C. Mitchell and Robin E. Jung Brown [eds.]. Urban Herpetology. Society for the Study of Amphibians and Reptiles.

Trenham, Pete and Chris Searcy. May 2016. Ecology of the tiger salamander workshop. Presentation.
An electronic copy of the slides from this presentation are available at: http://www.elkhornsloughctp.org/uploads/files/1463510183California%20Tiger%20Salamander%20Presentation%202016.pdf
This presentation covers the basic conservation biology of CTS.

Twitty, Victor C. 1941. Data on the life history of *Ambystoma tigrinum californiense* Gray. Copeia 1941 (1): 1-4.
Available via JSTOR.

This short paper discusses migration, spawning, larval development, and some early hybridization experiments crossing CTS with *Siredon* (now *Ambystoma*) *mexicanum*. The paper documents CTS migrating across Mayfield Road (now Junipero Serra Boulevard) from upland habitat to the Lagunita Reservoir and the road mortality that resulted.

U.S. Fish and Wildlife Service. 2016. Recovery Plan for the Santa Rosa Plain: *Blennosperma bakeri* (Sonoma sunshine)*; Lasthenia burkei* (Burke's goldfields)*; Limnanthes vinculans* (Sebastopol meadowfoam); California Tiger Salamander Sonoma County Distinct Population Segment (*Ambystoma californiense*). U.S. Fish and Wildlife Service, Pacific Southwest Region, Sacramento, California. vi + 128 pp.
An electronic copy of this draft recovery plan is available online at:
http://ecos.fws.gov/docs/recovery_plan/06012016_Final%20Santa%20Rosa_RP_signed_1.pdf

U.S. Fish and Wildlife Service. 2015a. Draft Recovery Plan for the Central California Distinct Population Segment of the California Tiger Salamander (*Ambystoma californiense*). U.S. Fish and Wildlife Service, Pacific Southwest Region, Sacramento, California. v + 53pp.
An electronic copy of this draft recovery plan is available online at:
http://ecos.fws.gov/tess_public/profile/speciesProfile.action?spcode=D01T

U.S. Fish and Wildlife Service. 2015b. Draft recovery plan for the Santa Barbara County Distinct Population Segment of the California tiger salamander (*Ambystoma californiense*). U.S. Fish and Wildlife Service, Pacific Southwest Region, Ventura, California. vi + 76 pp.
An electronic copy of this draft recovery plan is available online at:
http://ecos.fws.gov/tess_public/profile/speciesProfile.action?spcode=D01T

van der Ree, Rodney; Daniel J. Smith, and Clara Grilo (Eds.). 2015a. Handbook of Road Ecology. Wiley Blackwell, Chichester, UK.
This book is a reference on road ecology. The work was used to obtain definitions, the attributes of roads that might impact CTS, and information on the use of landscape genetics in road ecology.

van der Ree, Rodney; Daniel J. Smith, and Clara Grilo. 2015b. The ecological effects of linear infrastructure and traffic, p. 1 to 9. *In* Rodney Van Der Ree, Daniel J. Smith, and Clara Grilo (Eds.). Handbook of Road Ecology. Wiley Blackwell, Chichester, UK.
This chapter provides a succinct overview of the ecological impacts of roads, railways, and utility easements. The work was used to obtain definitions and the attributes of roads that might impact CTS.

Wilkinson, Samantha; Pamela Wiener, Alan L. Archibald, Andy Law, Robert D. Schnable, Stephanie D. McKay, Jeremy F. Taylor, and Rob Ogden. 2011. Evaluation for approaches for identifying population informative markers from high density SNP chips. BMC Genetics 12:45.
This paper discusses the development of SNP chips for cattle. A similar approach can be applied to CTS and hybrids. The key to this research was to develop chips with the most informative markers to determine the origin of individuals.

CTS Related Genetics Papers & Presentations

Fitzpatrick, Benjamin M. 2008. Dobzhansky-Muller model of hybrid dysfunction supported by poor burst-speed performance in hybrid tiger salamanders. Journal of Evolutionary Biology 21: 342-351.
Using the same eight genetic markers as used in other studies by Fitzpatrick and Shaffer the author noted a slower startle induced burst speed among BTS/CTS hybrid larvae than among CTS.

Fitzpatrick, Benjamin M. and H. Bradley Shaffer. 2004. Environment-dependent admixture dynamics in a tiger salamander hybrid zone. Evolution 58(6): 1282-1293.
This study was done at 12 breeding ponds in the Salinas Valley near Gonzales. Mitochondrial DNA and 8 nuclear markers were used. Salamander breeding habitat in the CTS and BTS hybrid zone may by roughly classified into: vernal pools, ephemeral cattle ponds, and perennial ponds. Nonnative alleles predominated in perennial ponds. There was a genetic difference between ephemeral cattle ponds and perennial ponds even if separated by short distances. This may be due to selection for some BTS alleles in perennial ponds.

Fitzpatrick, Benjamin M. and H. Bradley Shaffer. 2007a. Hybrid vigor between native and introduced salamanders raises new challenges for conservation. Proceedings of the National Academy of Sciences USA 104 (40): 15793-15798.
This study determined that BTS/CTS hybrid larvae showed increased survival in the ponds studied.

Fitzpatrick, Benjamin M. and H. Bradley Shaffer. 2007b. Introduction history and habitat variation explain the landscape genetics of hybrid tiger salamanders. Ecological Applications 17 (2): 598-608.
This study using interviews and eight markers (1 mtDNA and 7 nuclear DNA) looked at 28 ponds. Later the researchers selecting 3 markers for further study and sampled 85 ponds. They determined that much of the Salinas Valley contains a hybrid swarm of tiger salamanders. Nonnative allele frequencies tended to be lower outside of the Salinas Valley. Perennial ponds tend to favor hybrid salamanders. Translocation of BTS/CTS hybrids appears to be an important factor in the spread of introgression. Because only a small portion of the genome was sampled there is considerable uncertainty concerning the identity of pure CTS populations. The authors make recommendations concerning which populations should remain legally protected.

Fitzpatrick, Benjamin M., Jarrett R. Johnson, D. Kevin Kump, Jeremiah J. Smith, S. Randal Voss, and H. Bradley Shaffer. 2010. Rapid spread of invasive genes into a threatened native species. Proceedings of the National Academy of Sciences USA 107 (8): 3606-3610.
This research team (which includes both Fitzpatrick and Shaffer) used an expanded set of 68 single nucleotide polymorphisms (SNP’s) as diagnostic markers for CTS versus BTS/CTS hybrids. 65 of the 68 markers show a rapid transition to native alleles near the northern most introduction sites. However, 3 unlinked markers (E6E11, E12C11, and E23C6) show in high frequencies in ponds up to 94 km farther away. This indicates that introgression is likely to be more widely spread than previously recognized. Additionally it is possible that these markers are linked to genes that produce phenotypes with high adaptive value in California.

Johnson, Jarrett R., Benjamin B. Johnson, and H Bradley Shaffer. 2010. Genotype and temperature affect locomotor performance in a tiger salamander hybrid swarm. Functional Ecology 24: 1073-1080.

McCartney-Melstad, Evan; Benjamin Fitzpatrick, Jarrett R Johnson, Genevieve Mount, and H. Bradley Shaffer. Conservation genomics of the California tiger salamander (*Ambystoma californiense*): past, present, and future. California-Nevada Amphibian Populations Task Force January 8-10, 2014 (Abstract).
This conference presentation outlines the ongoing work to understand the CTS genome.

Shaffer, H. Bradley and Mark McKnight. 1996. The polytypic species revisited: genetic differentiation and molecular phylogenetics of the tiger salamander *Ambystoma tigrinum* (Amphibia: Caudata) complex. Evolution 50 (1): 417-433.
This was a phylogenetics study in which an approximately 849 base pair section of mitochondrial DNA was used to illuminate phylogeny and suggest putative species in the complex.

Shaffer, H. Bradley; Gregory B. Pauly, Jeffrey C. Oliver, and Peter Trenham. 2004. The molecular phylogenetics of endangerment: cryptic variation and historical phylogeography of the California tiger salamander, *Ambystoma californiense*. Molecular Ecology 13: 3033-3049.
This study examined the genealogical relationships of CTS across its range using mitochondrial DNA (mtDNA). Genetic samples were derived from salamanders collected from around the animal's range. CTS is a member of the tiger salamander species group which contains around 15 closely related taxa. However CTS is the sister taxon to the rest of the species from which it is deeply diverged presumably by vicariance during the late Miocene about 5 million years ago. The researchers identified four well supported mtDNA lineages that correspond to population discontinuities or potential major connectivity barriers. These are the Sonoma, Santa Barbara, southern San Joaquin Valley, and the Central Coast Range. Two less well defined clusters were discerned in the Central Valley and Bay Area.

Shaffer, H. Bradley; Müge Gidiş, Evan McCartney-Melstad, Kevin M. Neal, Hilton M. Oyamaguchi, Marisa Tellez, and Erin Toffelmier. 2015. Conservation genetics and genomics of amphibians and reptiles. Annual Reviews of Animal Biosciences 3: 113-138.
This paper provides a broad review of the conservation genetics of reptiles and amphibians. The BTS/CTS hybridization problem is specifically mentioned.

Wayne, Robert K. and H. Bradley Shaffer. 2016. Hybridization and endangered species protection in the molecular era. Molecular Ecology 25 (11):2680-2689 doi: 10.1111/mec.13642
This opinion paper presents an approach to determining which hybridized populations should be considered for protection under the endangered species act. Specific information is provided on and specific recommendations are made for CTS and BTS/CTS hybrids.

Web Resources

AmphibiaWeb**: Information on amphibian biology and conservation. [web application]. 2016. Berkeley, California: AmphibiaWeb. Available: http://amphibiaweb.org/. Ambystoma mavortium. http://amphibiaweb.org/cgi/amphib_query?where-genus=Ambystoma&where-species=mavortium**
(Accessed by Harold Hunt 24 June 2016).

Elkhorn Slough Coastal Training Program. Training. Ecology of the Tiger Salamander. **http://www.elkhornsloughctp.org/training/show_train_detail.php?TRAIN_ID=Ec99VYN** (accessed by Harold Hunt 16 June 2016).
A number of resources and papers on CTS are available for downloading at this website.

Gene Reviews [Internet] Illustrated Glossary Terms & Definitions http://www.ncbi.nlm.nih.gov/books/NBK5191/#IX-M (Accessed by Harold Hunt 21 July 2016)

Oxford Bibliographies. Storfer, A. 2015. Landscape genetics. Oxford Bibliographies in Evolutionary Biology. DOI: 10.1093/OBO/9780199941728-0066. http://www.oxfordbibliographies.com/view/document/obo-9780199941728/obo-9780199941728-0066.xml (Accessed by Harold Hunt 5 August 2016).

University of California. Division of Agriculture and Natural Resources. Ground squirrel best management practices.
http://ucanr.edu/sites/Ground_Squirrel_BMP/ (accessed by Harold Hunt 28 June 2016)

University of California, Los Angeles. The Shaffer Lab at UCLA. Publications. **https://www.eeb.ucla.edu/Faculty/Shaffer/publications.htm** (Accessed by Harold Hunt 15 June 2016).
This website provides access to the full texts of several papers on CTS and other genetic and genomic papers authored by those associated with this lab.

U.S. Fish and Wildlife Service. ECOS Environmental Conservation Online System. Species Profile for California Tiger Salamander (Ambystoma californiense). **http://ecos.fws.gov/tess_public/profile/speciesProfile.action?spcode=D01T** (accessed by Harold Hunt 15 June 2016).
This website provides access to Federal Government documents and notices related to CTS.

Appendices

Appendix 1 Glossary

This glossary contains definitions for terms which relate to hybridization taken from Allendorf et al. (2001), Allendorf et al. 2010, Perkel 2008, Morin et al. 2004 and Gene Reviews [Internet] Illustrated Glossary Terms & Definitions http://www.ncbi.nlm.nih.gov/books/NBK5191/#IX-M

Admixture: the production of new genetic combinations in hybrid populations through recombination.

Chip = DNA Microarray: A system using a plate covered with beads capable of combining with specific DNA sequences. Each bead is capable of combining with one specific DNA sequence. Each chip contains hundreds to thousands of beads each capable of combining with a different DNA sequence. The chip is automatically read to determine specific aspects of an organism's genotype. This is a rapidly changing technology which has become more capable and cheaper to use over the last few years.

Hybrid Swarm: a population of individuals that are all hybrids by varying numbers of generations. Hybrid swarms are formed when there is continued backcrossing among parent populations and the original (F_1) hybrids.

Hybrid Zone: an area of contact between two genetically distinct populations where hybridization occurs.

Introgression: gene flow between populations whose individuals hybridize.

Locus: A genome location, or marker, or base pair.

Marker: An identifiable segment of DNA (e.g. RFLP or microsatellite) with enough variation between individuals that its inheritance and co-inheritance with alleles of a given gene can be traced.

Microsatellite: A repetitive noncoding segment of DNA two to five nucleotides in length. Microsatellites are frequently used to discern genetic differences at the individual and local population levels.

Neutral Locus: A locus that has no effect on adaptation because all genotypes have the same fitness.

Proportion of Admixture: The proportion of alleles in a hybrid swarm or individual that comes from each of the hybridizing taxa.

Pure Population: a population in which there has been no hybridization and therefore contains only individuals from a single parental taxon.

Single Nucleotide Polymorphism (SNP): A nucleotide site in a DNA sequence where more than one nucleotide is present in a population and the variation is present in > 1% of the population. SNP is often pronounced as “snip”. SNP’s are commonly used in genetic and genomic testing to differentiate among population entities.

Appendix 2 Email Sent to Potential Interviewees

The following email was sent to the following people who were identified during the kickoff meeting as having knowledge about CTS: H. Bradley Shaffer, Christopher Searcy, and Sam Sweet.

Dear Dr. ______

I am with the California Department of Transportation’s Division of Research, Innovation and System Information. We are conducting a preliminary investigation into the feasibility of helping to manage the spread of introgression due to the hybridization of California tiger salamanders (CTS) and barred tiger salamanders (BTS) by using portions of the state highway system as barriers to movement. A preliminary investigation is a literature and practitioner review on a requested topic that provides an overview of existing research and best practices for the topic. Therefore we are seeking information regarding how CTS and hybrids move through the landscape.

Would it be possible to briefly communicate with you about this topic sometime over the next few weeks either by email or phone?

Caltrans's interest is in measures that we can utilize within highway rights-of-way to cost effectively limit salamander movement when that is required of us. Specific information we are interested in includes:

- In your opinion would it be useful to study the use the highway system as a means to manage the spread the spread of introgression by creating barriers to salamander movement?
- What genetic markers would be most useful for determining that a barrier is effective in limiting salamander movement?
- Are there differences between the ability of BTS/CTS hybrids and CTS to cross landscapes and man-made barriers?
- Could populations of introduced BTS (e.g. in Siskiyou County and near Clear Lake) be good surrogates for researching barrier effectiveness?
- What types of barriers, or combinations of barriers, would be most effective for preventing BTS/CTS hybrid movement across a highway (e.g. barrier fencing, short hydroperiod ponds, landscape vegetation, and rodent control).
- Is it feasible to try to block the movement of BTS/CTS hybrids while maintaining connectivity for other small animals?

My contact information is below.

Yours Truly,

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Appendix 3 Literature Search Methods

Staff searched the JSTOR and the EBSCO Greenfile databases available via the Caltrans transportation library using the following search terms:

- California tiger salamander full text
- Author names

Staff searched the TRID database available using the search term:

- Salamander

Staff searched the PubMed database using author names

Article abstracts were reviewed and pertinent articles were selected for further use. I also reviewed the literature cited sections of pertinent papers and the publications sections of author's websites to find other literature.
I used standard road ecology references and the extant literature on CTS to identify the pertinent attributes of roads to identify the attributes of roads that may impact CTS movement and survival in a landscape.

Acknowledgements

I thank the following for their considerable help with this PI:

- Dr. H. Bradley Shaffer, Dr. Samuel Sweet, and Dr. Christopher Searcy for their time and conversation about CTS.
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- Amy Golden and Nancy Siepel for their review of and comments on the original draft of this PI
- Susan Tyson of DRISI for overseeing the PI process.

All omissions and errors are my own.