Final Report

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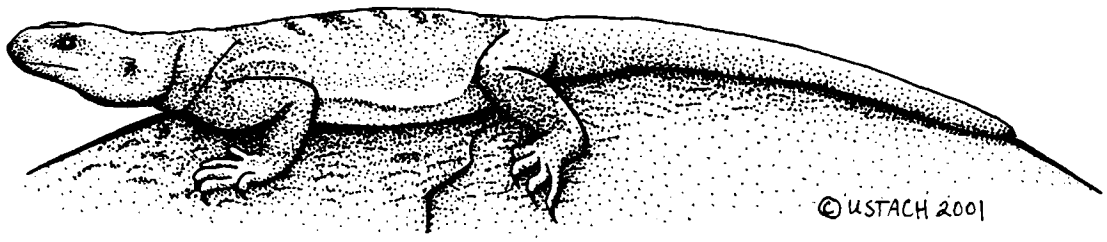
STATUS OF DISTRIBUTION, POPULATIONS, AND HABITAT RELATIONSHIPS OF THE COMMON CHUCKWALLA, *Sauromalus obesus*, IN NEVADA



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INTRODUCTION

As a primary consumer of vegetation in the desert, the common chuckwalla, *Sauromalus obesus* (*=ater;* Hollingsworth, 1998), is capable of attaining high population density and biomass (Fitch et al., 1982). The 21 November 1991 Federal Register (Vol. 56, No. 225, pages 58804-58835) listed the status of chuckwalla populations in Nevada as a Category 2 candidate for protection. Large size, open habitat and tendency to perch in conspicuous places have rendered chuckwallas particularly vulnerable to commercial and non-commercial collecting (Fitch et al., 1982). Past field and laboratory studies of the common chuckwalla have revealed an animal with a life history shaped by the fluctuating but predictable desert climate (Johnson, 1965; Nagy, 1973; Berry, 1974; Case, 1976; Prieto and Ryan, 1978; Smits, 1985*a*; Abts, 1987; Tracy, 1999; and Kwiatkowski and Sullivan, 2002*a, b*). Life history traits such as annual reproductive frequency, adult survivorships, and population density have all varied, particular to the population of chuckwallas studied. Past studies are mostly from populations well within the interior of chuckwalla range in the Sonoran Desert. Nevada is of particular interest because it includes the northwestern limit of their range in the Mojave Desert. In addition, little is known about the chuckwalla's resiliency to human mediated environmental disturbances and reduction in numbers; information that is crucial for management policies. In this report we provide the results of a state-wide chuckwalla distribution survey, characterization of habitat, population structure, and an experiment designed to model human mediated disturbances to chuckwalla populations. We then make recommendations based on the results towards the applied management of Nevada's chuckwalla populations.

BACKGROUND

What is a chuckwalla?

Chuckwallas are lizards that are commonly found in rocky outcrops that are usually situated on hills and mountains. The rocks provide areas for basking during the day and crevices for refuge from predators and temperature extremes. Chuckwallas are active during the day and use the crevices as a night time retreat. They are members of the scientific family Iguanidae. This family includes the more familiar Green Iguanas (*Iguana iguana*) of the American tropics and pet stores.

Chuckwallas can measure in length from the tip of the snout to the end of the vent up to 290 millimeters (~11 inches) and weigh up to 400 grams (~14 ounces)(this study). The tail, if undamaged, is more or less half the snout-vent length. The body and limbs are stout. The head, body and tail are depressed. The tail is thick and muscular at the base and then quickly tapers off to the tip. Sexual differences in appearance are pronounced (Kwiatkowski and Sullivan, 2002*a*). Mature males are larger, have wider heads, larger lower jaw muscles, and larger spiny scales on the sides of their necks than females and juveniles (Fig. 1). Adult males also have a larger and more prominent line of pores on the undersides of their thighs in comparison to females. Males excrete a waxy substance from these pores and deposit it on the substrate presumably as a signal to other chuckwallas. Chuckwallas have an approximate life span of 15–20 years (Abts, 1987).

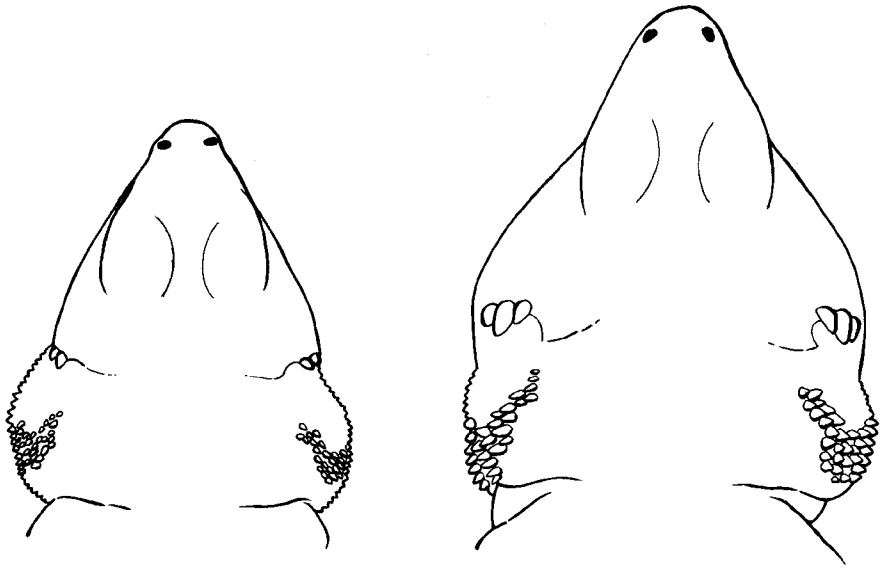


Figure 1. Dorsal view of the heads of a female (left) and male (right) chuckwalla. Mature males are larger, have wider heads, larger lower jaw muscles, and larger, spinier scales on the sides of their necks.

Color pattern varies greatly within and between geographic localities and between sexes (Shaw, 1945; Hollingsworth, 1998). Hollingsworth (1998) described color patter variation across the entire North American range based on the dorsal color pattern of the head, body, and tail of adult males. Although there was enough distinction among them for him to assign eight color pattern classes, he found there were no distinct borders among geographically adjacent groups. Color patterns gradually blend from one group to the next. Two Hollingsworth (1998) color pattern classes for chuckwallas are found within the borders of Nevada: the Northern Speckled Chuckwalla and the Virgin River Chuckwalla. Most chuckwallas in Nevada are in the Northern Speckled Chuckwalla class. This class includes chuckwallas from the northern portion of the range in California, Nevada, and Arizona (Hollingsworth, 1998). This color pattern class gradually intergrades in eastern Nevada into the Virgin River Chuckwalla of the Virgin River Gorge in southwestern Utah. Hollingsworth's (1998) descriptions for both classes are as follows:

> Northern Speckled Chuckwalla.—…adult individuals have dark brown to black speckling in the central dorsal region. A suffusion of light brown to brownish yellow circular markings occur across the body in some individuals, while others have black mottling on a light brown to brownish yellow ground color. This pattern class gradually intergrades into (another pattern class) to the south (page 135).
>
> Virgin River Chuckwalla.—Adult male Chuckwallas of the Virgin River Gorge… … are characterized by a dark brown to black head and the presence of 5 solid, dark brown to black transverse body bands with uniform light brown interspaces (pages 135 and 136).

Juvenile coloration is quite different and less variable than in adults. Juveniles and hatchlings have prominent black and white transverse bands on the body interspersed with white and red flecks or mottling. The contrast between the black and white bands on the tail is bolder than what is observed in adults.

History of the name

The lizard is commonly referred to in the United States as the chuckwalla or chuckawalla. Chuckwalla or chuckawalla is the English adaptation of the Spanish name, *chacahuala*; which in turn is derived from the Shoshone word *tcaxxwal* or *caxwal*, the word used by the Cahuilla Indians of southeastern California (Lawler et al., 1995). The northeastern Yavapai of Arizona call it *hamthul*, and the western Yavapai refer to the lizard as *sakowala* (Gifford, 1936).

The scientific name for the genus comes from the Greek, *Saura* (lizard) + *omalos* (level, flat), in reference to the broad flat body; the specific name comes from the Latin, *obesus* (stout, corpulent) in reference to the lizard's overall stout, pudgy appearance. Chuckwallas were given their first scientific name, *Sauromalus ater,* by Duméril in 1856. Working in France, Duméril based his description for the species on a preserved specimen sent to him from North America. The specimen had no specific locality data, but it was presumed to have come from off the coast of or islands in the Sea of Cortez. These were the places most likely the ship on the expedition from which the specimen came from would have landed. Two years later in 1858, Baird assigned the name *Sauromalus obesus* to chuckwallas from the interior mainland he determined to be a different species from *ater*. From then on, *S. obesus* was the name referred to all

chuckwallas living in the North American mainland deserts for 140 years until 1998. Hollingsworth (1998) determined that the specimen on which the original scientific description by Duméril (1856) is based is actually the same species as the specimens Baird distinguished as *S. obesus* in 1858. This means (according to the rules that govern the scientific naming of species) that all lizards referred to as *S. obesus* should be referred to as *S. ater* since *ater* was the first published scientific name. However, Montanucci et al. (2001) proposed the continued use of *S. obesus* regardless since it has been in use for so long (140 years) and is more familiar than the little used name *S. ater*. This report will continue to refer to chuckwallas as *S. obesus* for simplicity sake since most of the major studies of chuckwallas in the 20th century use *S. obesus*.

Where and when are chuckwallas found?

Chuckwallas are associated with the warm desert areas of the Mojave and Sonora that are primarily dominated by the creosote bush. Within Nevada, chuckwallas occur in suitable habitat in all of Clark County, the southern portion of Nye county, the southern half of Lincoln county, and the extreme southeastern tip of Esmeralda County. Chuckwalla distribution outside of Nevada includes the warm desert portions of southeastern California, the drainage of the Colorado from Glen Canyon Dam at Page, Arizona to the Henry Mts., Utah, western Arizona, southward to northwestern Baja California and Guayamas, Sonora, including several islands in the Sea of Cortez (Fig. 2). Chuckwallas are reported from sea level to around 1830 m (Stebbins, 1985).

Chuckwallas are active primarily from March to August. They emerge from hibernation in mid to late February when air temperatures reach the upper teens Celsius (mid 60's °F, degrees Fahrenheit; Berry, 1974). Lizards remain active out of crevices to bask and feed until air temperatures exceed 32–37 °C (88–98 °F; Berry, 1974). Activity begins earlier and ends later in the day as daytime temperatures increase with the season. Activity is greatest in the spring when the available standing crop of winter annuals is at its peak (Abts, 1987). The active season is essentially over by mid-August when annuals have dried up and potentially detrimental electrolyte concentrations exist in perennials (Smits, 1985*b*). Case (1976) concluded that chuckwalla winter inactivity resembles a true hibernation.

What do chuckwallas eat?

Dietary analysis by Shaw (1939), Sanborn (1972), Nagy (1973), Hanson (1974) and Prieto and Sorenson (1975*a*) indicate that chuckwallas only eat plant material (strict herbivores) under natural conditions in the wild. Some insects were found to be ingested but were a result of being unintentionally eaten with associated plant material. Mayhew (1963) and Prieto and Sorenson (1975*a*) found that some chuckwallas (especially juveniles) will accept and thrive on Meal Worms (*Tenebrio* larvae) in captivity.

Johnson (1965), Nagy (1973), Berry (1974), Case (1976), Abts (1987), and Kwiatkowski and Sullivan (2002*b*) all reported chuckwallas eat a wide variety of vegetation, but appear to prefer flower heads or moist leaves; and annual flowers (plants that only live for one season) are preferred over perennials (plants that live over several seasons) when available. In dry years when annuals are scarce or absent, lizards are able to subsist mainly on perennials.

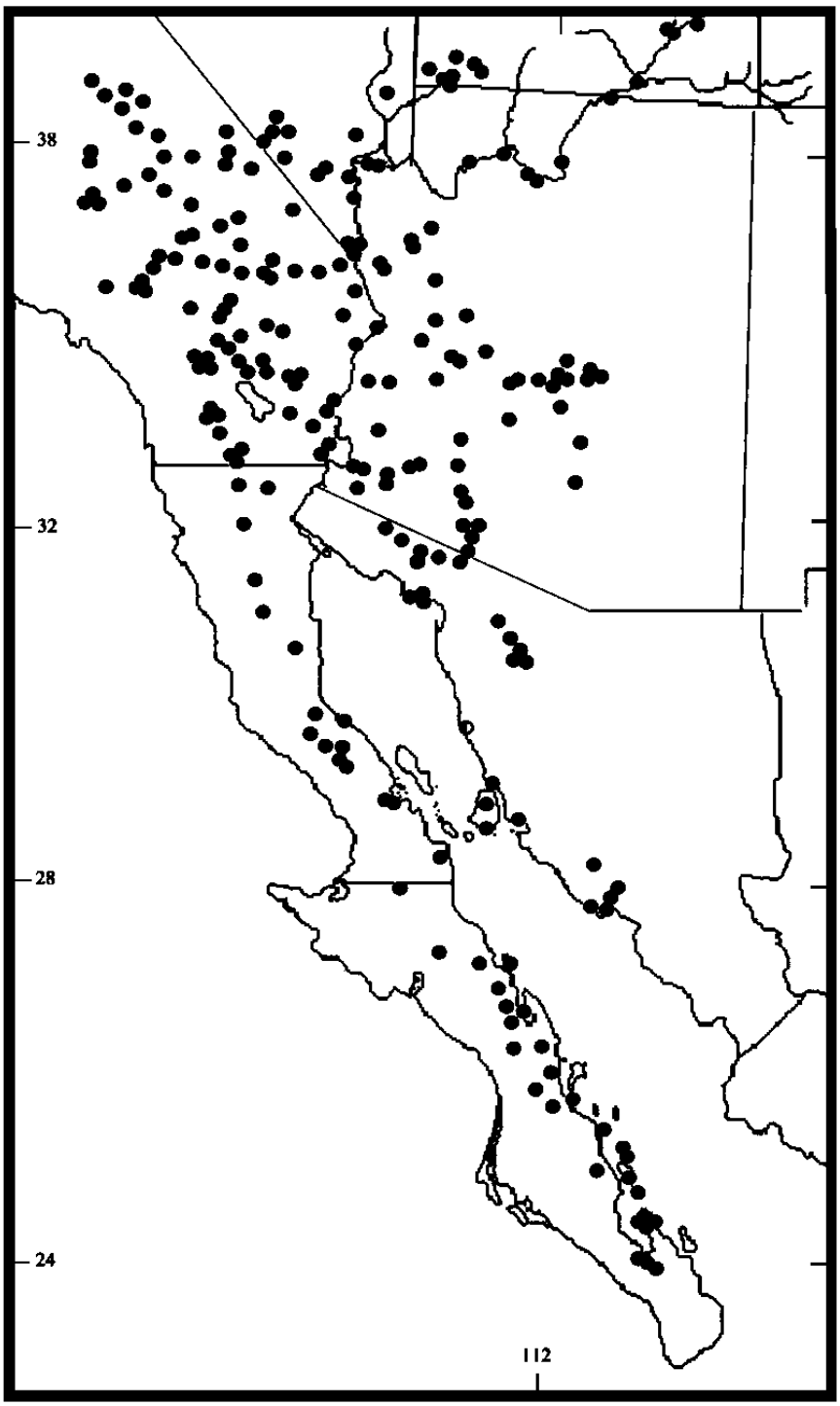


Figure 2. Dot-plot distribution of chuckwallas (*Sauromalus obesus*). Modified from Hollingsworth (1998).

What animals eat chuckwallas?

Observed instances of predation on chuckwallas are few. Berry (1974) observed chuckwallas respond to gliding birds overhead by retreating into crevices. Don Baepler (personal communication) noted chuckwalla remains around Peregrine Falcon (*Falco peregrinus*) nests studied by his graduate student Elise Schmidt. Prieto and Sorenson (1975*b*) reported to find two chuckwalla skulls directly below an American Kestrel (*Falco sparverius*) nest and a partially eaten male chuckwalla in the nest. They also observed a coyote (*Canis latrans*) carrying a dead chuckwalla. Berry (1974) found chuckwalla remains in coyote scat. Prieto and Sorenson (1975*b*) also found chuckwallas to defensively respond to post-anal gland secretions of Western Diamondback Rattlesnakes (*Crotalus atrox*), Kingsnakes (*Lampropeltus getulus*), and Gopher Snakes (*Pituophis catenifer*). A road killed Lyre Snake (*Trimorphodon biscutatus*, Barrick Museum specimen R6801) collected by Bill Cobb in Arizona "regurgitated a 12 cm section of *S. obesus* tail at death." The tail is preserved with the snake.

Physiology of chuckwallas

To avoid predation chuckwallas retreat into rock crevices and inflate their lungs to wedge themselves tightly within the crevice. Salt (1943) found that the lung capacity in defensive inflation was four times the normal inspiratory volume. Salt (1943) observed the rhythmic oscillations of the throat during defensive inflation and misleadingly described it as "swallowing air." Deban, et al. (1994) observed that the lungs are inflated by pulsatile contraction of the buccal cavity and more appropriately named this mechanism "pulse pumping." They noted that in pulse pumping air is forced down the glottis from the buccal cavity into the lungs (Deban, et al. 1994).

Norris and Dawson (1964) noted that chuckwallas expel fluid from the nostrils in the field by sneezing which explained why some lizards captured in the field have snouts encrusted with salt. He concluded that the salt-secreting gland appears to function in the excretion of high levels of potassium gained from the plants in their diet (Norris and Dawson, 1964). Templeton (1964) examined the bilateral nasal salt gland in chuckwallas and found it to play an important role as an "accessory kidney" to remove potassium with a small loss of water.

Norris and Dawson (1964) described the lymph sacs extending along the lateral abdominal folds to the head region. They speculated that the structures "may allow considerable expansion of the volume of extra cellular fluid during moist periods, thereby increasing the ability of these lizards to evade or defer deleterious effects of dehydration during periods when preformed water is scarce." However, Nagy (1972) found no evidence for water retention in the sacs. He noted that chuckwallas are dependent on succulent vegetation to maintain water balance and any excesses of water are excreted rather than stored. When succulent vegetation is no longer available in late spring and summer, chuckwallas avoid evaporative water loss by remaining in crevices most of the day, and avoid water loss associated with ingesting dry vegetation by not feeding. He mentions that this could be remedied by the drinking of rain water, but it not conclusive that chuckwallas drink regularly from standing water in the field (Nagy, 1972; Berry, 1974).

Behavior and reproduction in chuckwallas

Social systems vary between localities and appear to be dependent on availability of forage and population density. Johnson (1965) and Berry (1974) observed non-overlapping, larger home ranges and some territory defense in the males of their populations in the Mojave. Nagy (1987) observed no observable territory defense and the sexes had equal and overlapping home ranges in his population in the Sonoran Desert. Kwiatkowski and Sullivan (2002*b*) examined three populations in the Sonoran Desert and found female home range size to be related to food resources and male home ranges appeared to be related to female distribution, population density, and geology.

Chuckwallas are polygynous but not promiscuous (Berry, 1974). Males form a bond with a female that lasts for three and a half months. Males court each female in their home ranges almost daily from March through May but mate only in late May or early June (Berry, 1974). Kwiatkowski and Sullivan (2002*b*) observed male behavior consistent with territory defense polygyny (defending a site from other males while allowing overlap with multiple females).

Abts (1988*b*) found spermiation to occur in males in May and June. Abts (1988*a*) found males and females in his Sonora Desert population both reach reproductive maturity at about 125 mm snout-vent length and 2 years of age. Berry (1974) estimated that males and females in her Mojave population reach reproductive maturity at snout-vent lengths of 150 mm. Males reached this size in 3 years while females reached it in 5 years. Clutch size (Range=1–14) is highly correlated with body size (Abts, 1988*a*). Eggs are laid in late spring or summer. Hatchlings appear in early fall and have been observed to be active well into the winter months (Smits and Yorke, 1980).

The chuckwalla fossil record

No fossil material has been found for *Sauromalus* outside of their present day range. However, Avery and Tanner (1971) speculated that a fossil discovered in Wyoming and described by Gilmore (1928) as *Parasauromalus olseni* from the Middle Eocene, "may represent the ancestral stock of *Sauromalus*."

Chuckwalla bone fragments have been found from the analysis of layered mounds of well-preserved fragments of vegetation and bone fossils collected by rodents from the genus *Neotoma*, more commonly known as Packrats. These mounds are referred to as middens and are often found in caves and rock crevices throughout the arid southwest. Packrats collect bone-laden carnivore feces, parts of prey skeletons discarded by carnivores, or bones of small vertebrates that live in the rocks around or even use packrat dens as shelter (such as chuckwallas). Chuckwalla fossils from Nevada midden sites outside of the Colorado River trough have been dated at around 10,000 years before the present (Brattstrom, 1954; Norell, 1986; Hockett, 2000).

History of the scientific classification of chuckwallas

The genus *Sauromalus* was described by Duméril (1856) from the species *ater*. The description gave no type locality for the specimen. The species *obesus* was described, as *Euphryne obesus*, by Baird (1858) from Fort Yuma, California. In 1875,

Cope sunk *Euphryne* and synomonized *obesus* with *ater*. Schmidt (1922) revived the name *obesus* for chuckwallas from the southwestern United States and northern mainland Mexico. In 1945, Shaw designated the geographic variation contained in *Sauromalus obesus* by naming three subspecies. Based on his examination of new material from chuckwallas collected in intermediate localities, Shaw determined that the species status of *S. townsendi* proposed by Dickerson (1919) should instead be a subspecies of *S. obesus*. He designated *S. obesus townsendi* (Sonoran Chuckwalla) for chuckwallas found on Tiburon Island, Gulf of California, Mexico and the adjacent Sonoran mainland. Shaw also described the subspecies *Sauromalus obesus timidus* (Gila Chuckwalla) from southwestern Arizona and designated *Sauromalus obesus obesus* (Great Basin Chuckwalla) as those from southeastern California, southern Utah, northern Baja California, southern Nevada and Arizona north of the line Yuma-Casa Grande-Canyon Lake. Tanner and Avery (1964) named chuckwallas from the Colorado River area from Glenn Canyon Dam in northern Arizona, northward and eastward to just north of Hite in southern Utah as the subspecies *Sauromalus obesus multiforminatus* (Glen Canyon Chuckwalla).

Hollingsworth (1998) thoroughly examined specimens in the genus over the entire geographic range. His study included additional specimens that had been collected in intermediate localities during the fifty-three years since Shaw's analysis. He also examined the specimen from which *Sauromalus ater* was described and determined it was not distinct enough to be a different species from *S. obesus*. Based on scale counts and color pattern, he proposed that *Sauromalus ater* be recognized as a species composed of eight mainland and thirteen island populations. *Sauromalus obesus* and *S. australis* (mainland chuckwallas from peninsular Baja California) were all the same species. The subspecies designations for *S. obesus townsendi, S. o. timidus, S. o. multiforminatus,* and *S. o. obesus* should no longer apply. Results from his study indicated that a north-south cline exists for various characters. Northern chuckwallas are larger, have smaller scales and shorter tails while southern chuckwallas are smaller, have larger scales, and longer tails. While male color pattern can be very different in non adjacent geographic populations, they blend in adjacent groups (Hollingsworth, 1998).

History of chuckwallas as a human resource

Native Americans infrequently captured chuckwallas for subsistence. Gifford (1936) reported that the northeastern and western Yavapai extracted chuckwallas from rock crevices with a "sharp stick twisted into skin." Once extracted, they held the lizard by the tail and struck it against a rock to kill it. The lizard was then cooked either gutted or ungutted on hot coals. Jaeger (1950) observed southern Paiutes in southern Nevada preparing chuckwallas.

Steward (1941) documented the chuckwalla hunting techniques of Death Valley Native Americans. The hunting of chuckwallas was delegated to the women and children of the group. Small parties were formed early in the morning to collect the lizards mainly in the spring as they emerged from their crevices (Steward, 1941). Chuckwallas were also taken incidentally as women gathered plant foods, or when men were out hunting other game.

Wallace (1978) and Brown (1993) described and illustrated tools the Native Americans used to extract chuckwallas from their crevices. The tool consisted of a bone-

barbed hook at the end of a slender hardwood rod (Wallace, 1978). After the arrival of Europeans, wire instruments replaced the bone and wood instruments.

Steward (1941) reported that chuckwallas were sometimes traded to neighboring groups that came from areas where chuckwallas were scarce or did not occur. Whether the animals were traded dead or alive was not mentioned. The delivering of live chuckwallas across natural barriers of dispersal could have had an effect on the range and genetic structure of present chuckwalla populations.

Today, most chuckwallas are taken from the wild for the commercial pet trade and to a lesser extent, for science. Nevada, since the 21 November 1991 Federal Register (Vol. 56, No. 225, pages 58804–58835), listed the status of chuckwalla populations in Nevada as a Category 2 candidate for protection. Currently, Nevada is the only state that allows the collection of chuckwallas for the commercial pet trade. Most collectors begin collecting in March, and continue sometimes until the end of September. Most of the animals collected for the commercial pet trade are potentially breeding adults. Adult females and especially large dominant males fetch higher prices on the market than do juveniles (commercial collector, personal communication). Plus, juveniles do not survive the stresses of shipping and transporting as well as adults.

The Study Landscape: The Mojave Ecoregion of Southern Nevada

The Mojave Desert is situated between the Great Basin Desert to the north and the Sonoran Desert to the south. It is located in eastern California on the East side of the coastal Transverse Ranges and the Sierra Nevada between 34˚N and 37˚N latitude and in the southern one-sixth of Nevada, along the Virgin River drainage near St. George in southwestern Utah, and near Kingman in the northwestern corner of Arizona (MacMahon and Wagner, 1985; Rundel and Gibson, 1996). This study was conducted in the portion of the Mojave Desert contained within the southern borders of Nevada, which represents a large part of the northern most limit of chuckwalla distribution (Figures 3 and 4). This investigation is focused on populations of chuckwallas within the political borders of Nevada since the 21 November 1991 Federal Register (Vol. 56, No. 225, pages 58804–58835) listed the status of chuckwalla populations in Nevada as a Category 2 candidate for protection. Currently, Nevada is the only state that allows the collection of chuckwallas for the commercial pet trade. Commercial collectors concentrate most of their efforts in the southern tip of Nevada in the Newberry Mountains.

The geologic surface of the southern Nevada area is typical basin and range topography, formed by isolated mountain ranges oriented mostly along a north-south axis that are separated by broad basins and valleys. Extensive alluvial fans or bajadas, form around the perimeter of the mountains and extend outward to low-elevation intervening basins, which commonly contain dry lake beds that are also known as playas. Most of the ranges are fairly narrow, and in the study area the highest point is Charleston Peak 11,981 feet (3,652 meters), located west of Las Vegas, in the Spring Mountains. The elevation of the valley basins are usually above 1,968 ft (600 m) and seldom exceed 2,952 ft (900 m). The lowest point in the study area is along the Colorado River at around 574 ft (175 m), located near Laughlin. Most of the drainage in the study area is internal within the basin and range topography, except for the Muddy and Virgin rivers and several other smaller drainages (for example, Las Vegas Wash), which drain into the Colorado River. The Colorado subdivision of the Sonoran Desert reaches it's apex in the southern most tip of

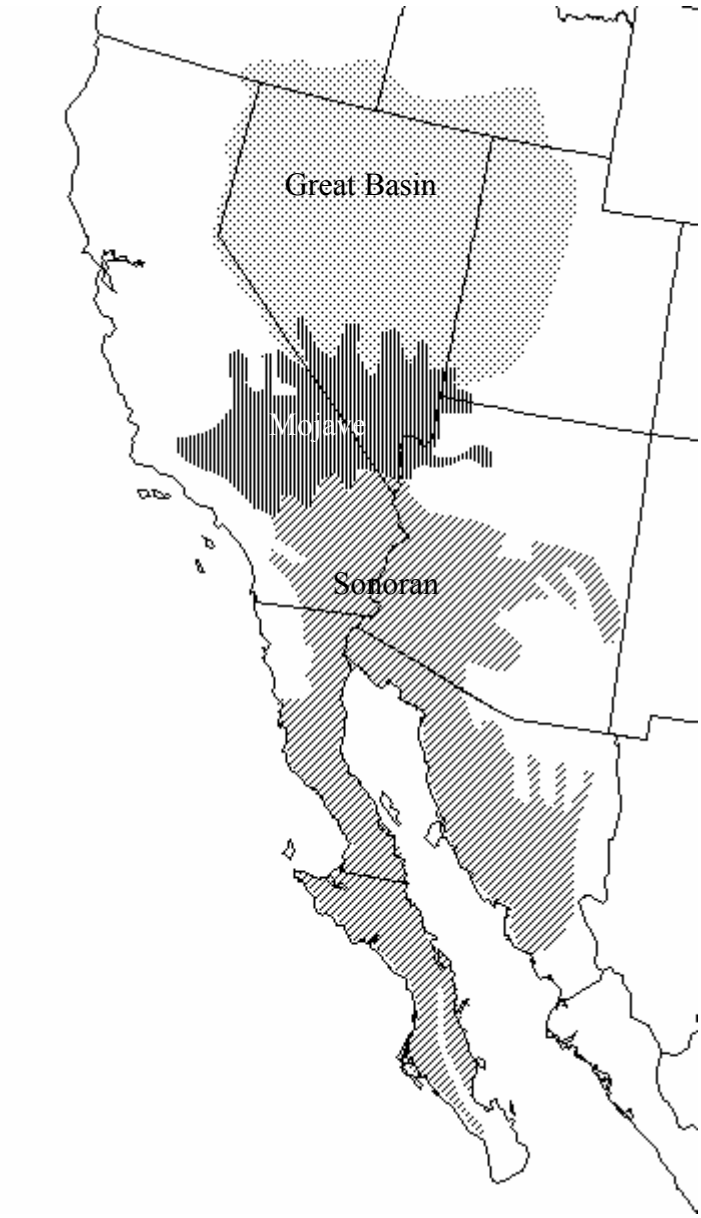


Figure 3. Spatial distribution of the Mojave Desert between the Great Basin Desert and the Sonoran Desert.

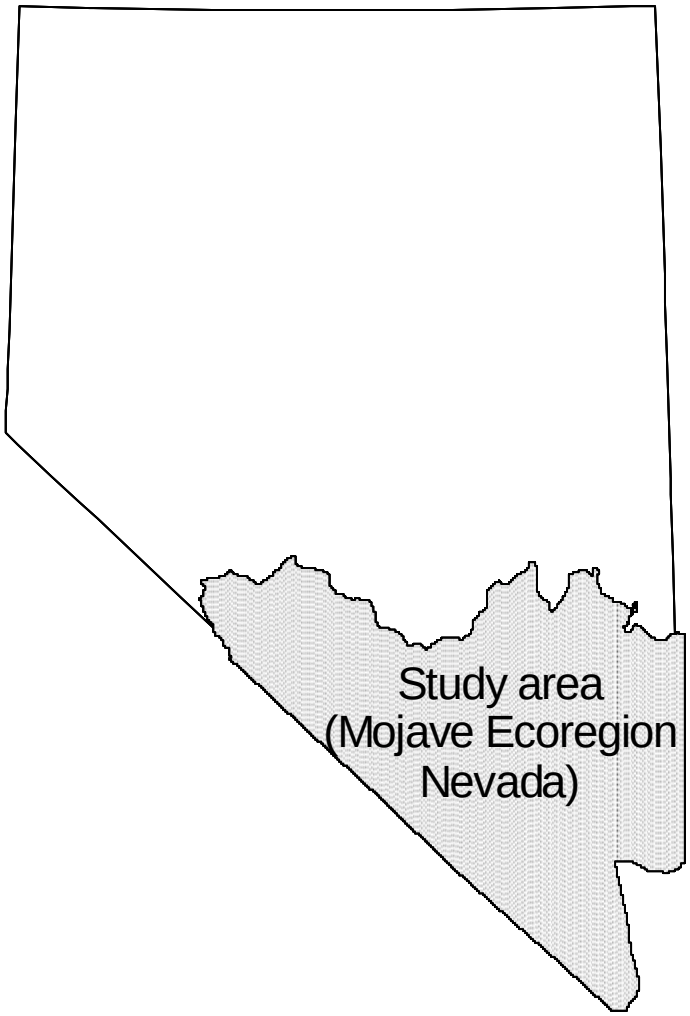


Figure 4. Distribution of the study area in the portion of the Mojave Desert contained within the southern borders of Nevada

Nevada along the Colorado River trough and reaches west to include the Newberry Mountains. However, this boundary is ill defined and admittedly arbitrary (Rundel and Gibson, 1996). This is the northern most extension where characteristic Sonoran species begin to appear, such as Ocotillo (*Fouquierie splendens*) and intermix with characteristic Mojave species such as Joshua tree (*Yucca brevifolia*). Thirty percent of the total flora (127 species) surveyed in the Newberry Mountains illustrate affinity with the Sonoran Desert (Holland, 1982).

Weather

Most of the precipitation within the study area falls in the winter and spring. This results when the northeastern Pacific High is displaced to the south, and low pressure troughs form over the western United States (MacMahon and Wagner, 1985). Under these conditions, large cyclonic storms from the Gulf of Alaska may bring in moist and unstable air masses. Most of the moisture in these air masses is dropped against the western slopes of the Sierra Nevada or Transverse Ranges. As the air masses cross the Mojave Desert they are further reduced of moisture by compressional heating of air descending into the desert basins. Occasionally, a storm or rapid succession of storms sweep through and bring light to moderate fall and winter precipitation for one to several days (MacMahon and Wagner, 1985). These storms bring cool temperatures, gusty winds, and sometimes snow in the high mountain elevations.

Summer precipitation arises less reliably from a totally different weather system (MacMahon and Wagner, 1985). Summer precipitation occurs when the subtropical high is weakened, resulting in the monsoonal influx of moist air from either the Gulf of Mexico or the Gulf of California. Thermal heating of these humid air masses produces strong conventional storms bringing brief but intense rain to local areas.

These two types of seasonal precipitation define the rainfall patterns of the three warm desert regions found in the southwestern United States (MacMahon and Wagner, 1985). The Chihuahuan Desert in the east receives mostly summer precipitation, and the Mojave Desert in the west receives mostly winter precipitation. The Sonoran Desert located between the two receives both. This biseasonal rainfall typically occurs more reliably in the southernmost part within the study area in the Newberry Mountains, while the majority of the precipitation in the northern areas arrives in the winter.

Total mean precipitation levels show a strong correlation with elevation, with areas below 3280 ft (1000 m) ranging from 3.5–4.7 inches/year (90–120 millimeters/year) and a general linear increase from 4.7 to 15.4 in/yr (120 to 390 mm/yr) between 3280–6562 ft (1000–2000 m) (Rundel and Gibson, 1996).

The northern area of the study has mean summer high (daytime) temperatures reaching 99˚F (37˚C) in July, but mean nighttime temperatures can drop to 60˚F (16˚C) because of cloudless skies. Near the base of the Newberry Mountains near the Colorado River, the mean summer high (daytime) temperatures reach 108˚F (42˚C) in July with comparatively less cooling at night than in northern portions because of increased humidity.

Plants

The flora of the lowland areas of the study area in the north is one that is not clearly defined on the basis of endemic species or distinctive in having a high diversity of perennial plants (Rundel and Gibson, 1996). The flora above the study area is typical perennial plant species from the cold-adapted Great Basin (MacMahon, 1979). Characteristic species of the Colorado River area near the Newberry Mountains are also present in the somewhat warmer western Sonoran Desert (Holland, 1982). The area between can be characterized as transitional between the two provinces (MacMahon, 1979).

The Study

The goals of this study are to field test the distribution model completed in 1995 that characterizes how chuckwallas are distributed within Nevada, characterize the habitat in which they are found, develop a relative abundance index, conduct an experiment designed to model human mediated disturbances, determine the genetic relationships among populations, and make management recommendations based on the results.

CHUCKWALLA DISTRIBUTION IN NEVADA

Determining Distributions

Historically, determining species distributions has been done by developing dot distribution maps and range maps. Known localities are taken from data associated with voucher specimens and then plotted on a map of the region of interest (see Fig. 2). Species range boundaries are drawn around the locality points based on the particular researcher's expertise and personal knowledge of habitat for the species in question. While dot locality maps are probably the most objective, they are limited in their scope of application when designating the range of a species in relation to habitat. Boundaries can vary among investigators since weights of boundaries among dot localities are based on the particular individual's expertise and knowledge.

Geographic Information Systems (GIS)

Geographical information systems use the same locality data and individual expertise, but delimitate boundary limits between points by less subjective means. Boundaries among dot localities are based on statistical evidence linking ecological processes to assess species distribution across landscapes. GIS models apply a modern, spatially explicit approach to predicting species distribution based upon habitat characteristics.

GIS are computer-based information systems which enable the capture, modeling, manipulation, retrieval, analysis and presentation of geographically referenced data (Worboys, 1995). They are now standard tools in biological disciplines that are interested in viewing or analyzing spatial or map based data. GIS has been used for a variety of investigations including locating biodiversity hotspots (Jones et al., 1997; Noonan, 1999),

identifying "gaps" in biological diversity protection (Edwards and Scott, 1994), and modeling metapopulation viability (Lindenmayer and Possingham, 1995). The most common use is for habitat and species distribution mapping to help answer questions about conservation and management, for example of Florida panthers (*Felis concolor*) (Maehr and Cox, 1995), Black-tailed Jackrabbits (*Lepus californicus*) (Knick and Dyer, 1997), Sage Grouse (*Centrocercus urophasianus*) (Homer et al., 1993), and communities of birds in remote areas (Lenton et al., 2000).

This aspect of the project has been designed to provide information on distribution and regional habitat of chuckwallas and to identify areas of high probability of chuckwalla presence to aid in the survey. We concentrated our efforts towards testing the map that was delivered according to contract in the 1995 report (see Figure 14 therein) and exploiting any opportunities to collect microhabitat data as it arose.

Field testing the 1995 model

We tested the predicted distribution of chuckwallas predicted by the model developed in 1995. First, 50 random locations were chosen. The number of sites chosen was determined by manpower restrictions. To select the 50 locations, we obtained x, y coordinates for each cell in a grid containing the predicted sites by the model. We then used the Random Sample routine in S-Plus (2000 Professional release 1, Mathsoft, Inc.) to pull out 50 random sets of x, y coordinates from the list. Each coordinate pair had an equal chance of being selected.

To test the GIS predictive model, 40 of the 50 random areas were located on the ground and searched during the spring and early summer of 2000 to determine the presence or absence of chuckwallas. While the survey in 1995 was conducted largely by sampling potential sites near roads and jeep trails, the survey to field truth the refined model was not as simple. Since sites were selected randomly by computer algorithm, a considerable number of sites could only be sampled by long hikes on foot and off trail across rough desert terrain. Field logistics allowed us to only sample 40 sites. A Trimble™ Scout Global Position System unit was used to navigate to each selected site. We documented sites surveyed for chuckwallas by first photographing the site. Since information from the literature and from experts knowledgeable about chuckwalla habitat indicate that chuckwallas are most likely encountered in rocky outcrops that provide adequate crevices for hiding, our sampling focused on hillsides that contained rocky outcrops. For the sampling purposes of the survey, hillsides that contained rocky outcrops were designated suitable habitat. Presence/absence of chuckwallas was determined by a visual survey of selected hillsides with rocky outcrops. A hillside was surveyed for chuckwallas by setting up a spotting scope approximately 150 m away from the face of the hillside. Because the average home range size of chuckwallas is approximately 10 hectares (Johnson, 1966; Berry, 1974), a 100 x 100 m (~10 ha) grid was establish on a hillside that contained rocky outcrops and systematically scanned for 15 minutes. Presence of chuckwallas was confirmed by sight. Since chuckwallas are wary and easily scared into crevices even from approaches of great distances (Miller and Stebbins, 1964), an attempt was made to detect chuckwallas after the initial focal scan by searching the rocks on foot for scat for an additional 15 minutes. Presence or absence was straight forward and reliable since areas inhabited by chuckwallas contain conspicuously placed droppings (elongate cylindrical pellets containing plant fibers),

which mark basking sites and favored retreats (Stebbins, 1985). Time spent scanning hillsides for chuckwallas and scats per unit area were standardized for each site (15 min/10,000 sq. m). The probability level of correctly predicting use areas was expressed as a percentage of the number of sites containing chuckwallas or chuckwalla sign to the total number of sites (40).

Of the sub-sample of 40 predicted chuckwalla sites in the refined model, chuckwalla presence was confirmed at 35 locations (86% probability of predicting use areas). Stoms et al. (1992) proposed various factors regarding habitat maps that could be sources of uncertainty, and thus could affect the sensitivity of a GIS analysis. A great source of uncertainty is reliability in the GIS output product due to errors and uncertainties in data inputs (Stoms et al., 1992). Some of these uncertainties likely contributed to the discrepancies between slope values in the model and values found in the field. Observation data are subject to many sources of uncertainty, such as the accuracy of their location coordinates and the resolution of coverage. For example, the relatively gentle slopes that successfully predict chuckwalla occurrence are deceiving. These gentle slopes reflect the poor resolution of slope in a biologically meaningful area for chuckwallas. Elevation, slope, and aspect were estimated from 90 m elevation grid data. The majority of the localities during the 1995 survey were recorded with a GPS at the point where mountain ranges rise abruptly out of the surrounding valley. Four of the five incorrectly classified sites were on bajadas that had a slope within the range of the model (the other incorrectly classified site was on a hillside with no rock outcrops). The slopes entered into the model at these points are a combination of the slope of the mountain and the adjacent flat. While the model generally predicted this biological meaningful habitat with these slope values, they did not reflect the greater slopes found in the field at the microhabitat level. Essentially, most of the predicted localities in the model are located where the rocky slope of the mountains comes into contact with the beginning of the gently sloping bajada. This is also the area where one is most likely to find chuckwallas in the field. Chuckwallas are more abundant in areas where they have access to soil soft enough for females to dig nests to lay their eggs (Johnson, 1965). In addition, these areas might be more attractive since they harbor additional species of spring or summer annual plants for chuckwallas to exploit (Abts, 1987). Thus, the use of updated coverages with greater resolution and possibly additional map layers (e.g., exposed rock) could increase the sensitivity of our analysis in predicting suitable habitat for chuckwallas.

While it may be intuitive to any savvy desert herpetophile that chuckwallas are essentially on almost every rocky habitat (and therefore the need to model is superfluous), it is precisely for this reason that they make a good model organism. The fact that they have well defined habitat requirements and presence/absence confirmation is unambiguous makes model building and testing more strait forward. The model characterized chuckwalla habitat as patchily distributed both within and among mountain ranges, with flat valleys and bajadas and high elevation sites being unsuitable. Within the predicted areas, certainty improves if one can ascertain the presence of suitable crevices. Used alone, this model was very successful at providing a broad landscape picture of the patchy nature of chuckwalla habitat. Once within predicted suitable habitat, as identified from the GIS map, further analysis of the habitat on a smaller scale (Gabler, 2000) may be necessary to determine fine scale factors that may be a more accurate predictor of suitable chuckwalla habitat (such as characteristics of preferred crevices in rocks).

Chuckwallas likely select areas for use on a smaller scale than can be completely provided for by this GIS model. This model, however, can be a very useful first step when trying to assess any discernable patterns of chuckwalla habitat over a landscape at a broad scale and to identify any gaps in their distribution and if these gaps explain any possible genetic substructuring. This model is a useful tool to visualize the distribution pattern of chuckwallas and to characterize the patchy nature of their distribution among mountain ranges in southern Nevada. The ability to accurately predict suitable chuckwalla habitat increases with analysis of the habitat (as identified from the GIS map) on a smaller scale in the field.

CHUCKWALLA HABITAT IN NEVADA

After getting a broad scale characterization of chuckwalla distribution using the GIS model, it is necessary to collect fine scale data at known chuckwalla localities in the field. We collected data on elevation, slope, aspect, perch site to crevice distance, and perch site to perennial distance. The point quarter method was used to collect the perennial plant data with crevice as the center point. This information was tabulated on five lizards per site (presuming five or more lizards were located). Microhabitats where lizards are not found were not measured because we are attempting to describe the relationship between chuckwalla presence and habitat characteristics. It is not useful for this model to measure where they don't occur.

Raw data collected in the field at a finer a scale for 82 chuckwallas are found in Appendix A. The distances chuckwallas traveled from basking sites to crevices for cover ranged from zero to 12 meters. The mean distance was 1.04 meters (±1.7 meters). This relatively short distance is not surprising since chuckwallas are large bodied ectothermic herbivores and require relatively more time in the sun to bask to reach their active temperature than do other lizards (Zimmerman and Tracy, 1989). Before they reach their optimal active temperature, chuckwallas may be vulnerable to predators so they don't venture far from a safe retreat. While the map identified areas of slope and aspect that most likely contain rocky outcrops, it is necessary for habitat to contain suitable crevices for chuckwallas. While any savvy desert herpetophile may know a suitable crevice when they see one, the actual suitability of a crevice is hard to quantify. The model in the map is to be used as a sample area and ultimately the suitability must be checked in the field (although the model was 86% accurate in predicting).

The mean distance to the nearest perennial plant and crevices in which chuckwallas were found or observed retreating into was 8 ft (±7.5 ft) or 2.5 m (±2.3 m). Distances ranged from near zero to 72 ft (22 m). This characterizes another aspect of microhabitat quality. Chuckwallas are commonly found in habitat with some perennial vegetation.

While the GIS model characterized predictive habitat in south east aspects at a broad scale, the surface of chuckwalla basking sites within the broader hillside were most commonly south facing (mean=181˚; standard deviation=36˚; range=130˚–264˚). This makes biological sense in that the greater surface area of the hillside in general is most likely to receive warming morning sun rays early in the season and less likely to receive direct sunlight when radiation is at its greatest in the summer season. Chuckwallas were most often found basking in positions that faced south within the greater south eastern hillside.

As discussed earlier, the GIS model underestimated the slopes but correctly localized habitat near the outcrop and flat interface. Values taken in the field at sites for chuckwallas were steeper (mean=26˚; standard deviation=5.6˚; range=18–36). The likelihood of detecting chuckwalla presence in the field is greatest at the interface where mountain ranges rise abruptly out of the surrounding valley. Chuckwalla abundance appears to be a function of the quality of the rocks that contain suitable crevices for retreat and rock piles that provide basking sites. It appears that the populations in the Newberry Mountains may harbor the greatest densities of chuckwallas per unit area compared to populations in other mountain ranges in Nevada.

CHUCKWALLA ABUNDANCE INDEX

Measuring population abundance requires choosing some arbitrary boundaries, counting the individuals within the boundaries by visual census, and then dividing by the area. Observations in the field indicate that the number of chuckwallas observed basking in the early morning hours before they become active may be a useful index to estimate the abundance of chuckwallas present in the boundary. Abundance commonly varies over a species range, from high values where local conditions are most conducive to survival and reproduction to zero where these conditions don't exist. Therefore, we attempted to include known chuckwalla populations from geographically disparate parts within their range in Nevada. We counted the number of chuckwallas within a defined boundary and then compared this number to the total number of animals subsequently removed from the site in order to develop a useful method of estimating local chuckwalla population abundance.

Study Populations

Three geographically distinct locations in Nevada were selected for a series of observation trials and subsequent absolute counts through specimen removal at each site. A total of nine isolated populations were used; three each in three broad geographic locations. The three broad geographic locations include: 1) one that represents the Colorado Desert in southernmost Nevada, 2) one that represents the northeastern fringe of chuckwalla distribution in the Mojave Desert in Nevada; and 3) one that represents the northwestern fringe of chuckwalla distribution in the Mojave Desert in Nevada.

Each site was chosen so that it was large enough to host a chuckwalla population meaningful to relative abundance goals, yet small enough in area to allow practical attainment of goals. Each of the populations occupied an uplift roughly 200 meters square and that was bordered by unsuitable habitat on all sides at least for 100 meters and less than 1 kilometer. We considered sixteen sites as potential sites for the study in an early reconnaissance. Among the sixteen possible sites we chose nine sites that best fit the criteria. Each occupies an uplift roughly 200 meters square and that is bordered by unsuitable habitat on all sides at least for 100 meters and less than 1 kilometer. Each have south-west facing slopes that contain suitable rocky outcrops large enough to incorporate the 100 meter square sampling unit.

The sites incorporate two of the three broad geographic locations mentioned in the proposal: one represents the Colorado Desert in southernmost Nevada; and one represents

the northwestern fringe of chuckwalla distribution in the Mojave Desert in Nevada. The third region proposed to represent the northeastern fringe of chuckwalla distribution in the Mojave Desert in Nevada was not established exactly on the fringe of chuckwalla distribution. It was established more in an east-central location because only two sites that fit site criteria could be found in the northeastern region. The east central location is never the less intriguing since it is isolated geographically by the Virgin and Colorado Rivers from all other Nevada populations.

What follows is a list of sites.

Newberry Mountains (NB), Colorado Desert geographic region.
1:100,000 scale Davis Dam map.

NB-1: T. 32 S., R. 65 E. sec. 11. (Bridge Canyon 7.5' Quadrangle).
N 35˚10.375', W 114˚41.990'.
NB-2: T. 32 S., R. 65 E. sec 36 (Mt. Manchester 7.5' Quadrangle).
N 35˚06.373', W 114˚42.581'.
NB-3: T. 33 S., R. 65 E. sec. 3 (Mt. Manchester 7.5' Quadrangle).
N 35˚07.208', W 114˚41.288'.

Bonnie Claire (BC), Northwestern Mojave geographic region.
1:100,000 scale combined Last Chance Range and Pahute Mesa maps.

BC-1: T. 9 S., R. 43 E. sec. 17 (Bonnie Claire Lake 7.5' Quadrangle).
N 37˚08.977', W 117˚10.827'
BC-2: T. 15 S., R. 50 E. sec. 24 (Striped Hills 7.5' Quadrangle).
N 36˚37.580', W 116˚17.671'
BC-3: T. 15 S., R. 50 E. sec. 25 (Skeleton Hills 7.5' Quadrangle).
N 36˚37.282', W 116˚17.945'

Virgin Mountain (V), East central Mojave geographic region.
1:100,000 scale Overton map.

V-1: T. 16 S., R. 70 E. secs. 21, 22 (Whitney Pocket 7.5' Quadrangle).
N 36˚31.870', W 114˚09.928'
V-2: T. 16 S., R. 70 E. sec. 22 (Whitney Pocket 7.5' Quadrangle).
N 36˚31.795', W 114˚09.578'
V-3: T. 16 S., R. 70 E. sec. 22 (Whitney Pocket 7.5' Quadrangle).
N 36˚31.710', W 114˚09.626'

Baseline Focal Sampling

The number of animals observed in this aspect of the study was then used as a basis for developing a density index based on the number of animals observed versus the number of animals removed from the site (described in detail below). We calculated the baseline number of animals at each site as follows. First we calculated the median number of animals observed for each day during the four hour observation period. This was done for three days at each site. These three median numbers were then averaged to

obtain the baseline number of animals observed for each site. This number was then used to roughly predict the total number of animals present at each site.

Each population was sampled by spotting scope in the manner used in the 1995 statewide distribution study. Areas 100 x 100 meters were scanned for one hour a total of 12 times per site (four times per day for three days) to measure daily variance in the lizard numbers observed. The three days were at least ten days apart. Counts of lizards were conducted in the morning (8AM–12PM) from March through May, the peak activity period for chuckwallas. This sampling only can be done on days when the weather conditions are suitable for lizard activity.

Only three sites per year were planned to be visited, one in each of the three geographically distinct locations. Visitation to all nine sites was staged over three springs. In 1997 we concentrated our efforts on V-1, NB-3, and BC-1. In 1999 we concentrated our efforts on V-3, NB-1, and BC-2. In 2000 we concentrated our efforts on V-2, NB-2, and BC-3.

Observation trials began with the geographic location that represents the southern fringe of chuckwalla distribution in the Mojave Desert in Nevada (Newberry Mountains) and then moved towards the sites on the northern fringe of their distribution.

Removal

After all populations were sampled as described above, we attempted to remove all chuckwallas from each site for the remainder of each respective season. In 1997 we concentrated our efforts on V-1, NB-3, and BC-1. In 1999 we concentrated our efforts on V-3, NB-1, and BC-2. In 2000 we concentrated our efforts on V-2, NB-2, and BC-3. The utmost of care was given to collect live and unharmed specimens by hand. Assistance in the field during the various stages of the removal aspect was provided by Steve Clements, Robert Espinoza, Kim Field, Christine Foley, Daniel H. Foley III, Stephanie Gardner, Ty Gardner, Jesse Meik, Dr. Joseph R. Mendelson III, Jeffrey E. Motychak, Daniel G. Mulcahy, Ken Nussear, Kirk W. Setser, Eric Simandle, Allen Spaulding, Craig Steele, Chris Tracy, Dr. Richard C. Tracy, and Paul C. Ustach. The majority of the chuckwallas were removed within the first week of collecting. Most of the chuckwallas were collected by the teams the first day at the sites and numbers obtained per day declined steadily from the first day to essentially none after three days. Effort was taken to assign equal numbers of personnel on the teams for each outcrop. Care was taken to spend equal time searching on each outcrop.

Most of the animals were collected by extracting the animals from rock crevice retreats by prodding them out with a metal dowel. Minimum damage to animals and habitat was a great priority.

Specimens were measured (mass, size, sex) at the site of capture and retained dead and/or alive indefinitely. Some animals were transported to Dr. Tracy's lab at the University of Nevada at Reno. Dr. Tracy maintains a superbly cared for colony of captive animals for diet and behavior research. Animals that were accidentally killed or died in captivity were preserved and deposited at the University of Texas at Arlington Collection of Vertebrates.

Fecal pellets, or chuckwalla droppings, are identified as distinctive elongate cylinders that contain nothing but plant fibers. Field observations indicate that there are rocks within the greater outcropping that contain a greater amount of fecal pellets relative

to other rocks. These rocks may be preferred fecal deposition sites (latrines) or they are simply associated with favored basking sites or crevices. A latrine was defined as a protruding rock or group of rocks that had a noticeable amount of fecal pellets on the surface of the rocks and filled spaces between rocks. We removed all fecal pellets from the sites and searched for fresh droppings in subsequent seasons to determine recent chuckwalla activity. We didn't remove any new pellets subsequently, only observed. We used these observations only as a meter to detect presence or absence of chuckwallas in evaluating the success of removing most of the chuckwallas from each study site.

Activity of Chuckwallas

Table 1 shows the number of animals observed for the days chuckwallas were surveyed in 1997, 1999, and 2000. Animals begin to come out of their crevices at air temperatures around 70°F (21°C). They remain at one perch (usually a projecting rock) for around two hours or until air temperatures reach around 97°F (36°C). After this period of basking they begin to move. Although animals can be found basking earlier and at cooler temperatures, most are not out of their crevices yet. By the time air temperature is greater than 86°F (30°C) animals begin to move about and are harder to count.

Table 1—Number of lizards observed for the days chuckwallas were surveyed.

1997	NB-3	7-Mar-97	
	time	temp (°C)	#chucks
	8:00	19	
	8:15	20	
	8:30	20	
	8:45	19	
	9:00	20	
	9:15	23	
	9:30	23	2
	9:45	23	2
	10:00	26	2
	10:15	25	3
	10:30	26	3
	10:45	28	2
	11:00	27	2
	11:15	28	4
	11:30	29	2
	11:45	28	2
	12:00	29	2

Table 1 (continued)

NB-3 time	8-Mar-97 temp (°C)	#chucks
8:00	18	
8:15	21	
8:30	21	4
8:45	24	4
9:00	23	4
9:15	24	6
9:30	22	5
9:45	24	5
10:00	24	4
10:15	27	3
10:30	26	3
10:45	27	2
11:00	29	
11:15	25	
11:30	29	1
11:45	29	1
12:00	28	1

NB-3 time	9-Mar-97 temp (°C)	#chucks
8:00	21	1
8:15	21	1
8:30	21	2
8:45	22	5
9:00	25	5
9:15	24	4
9:30	24	4
9:45	24	4
10:00	25	4
10:15	25	4
10:30	25	5
10:45	27	2
11:00	28	1
11:15	26	
11:30	27	
11:45	28	2
12:00		

Table 1 (continued)

V-1 time	10-Mar-97 temp (°C)	#chucks
8:00	19	
8:15	19	
8:30	22	
8:45	21	
9:00	25	1
9:15	21	1
9:30	23	
9:45	23	
10:00	24	1
10:15	22	2
10:30	24	1
10:45	25	
11:00	26	1
11:15	26	2
11:30	24	2
11:45	27	3
12:00	28	4

V-1 time	11-Mar-97 temp (°C)	#chucks
8:00	20	
8:15	23	2
8:30	25	2
8:45	24	3
9:00	23	3
9:15	22	4
9:30	26	5
9:45	23	4
10:00	25	1
10:15	29	4
10:30	25	2
10:45	26	3
11:00	31	5
11:15	27	6
11:30	27	6
11:45	32	2
12:00	30	2

Table 1 (continued)

V-1	12-Mar-97	
time	temp (°C)	#chucks
8:00	26	
8:15	27	
8:30	27	3
8:45	26	6
9:00	24	4
9:15	28	4
9:30	27	4
9:45	30	5
10:00	32	3
10:15	29	3
10:30	31	2
10:45	30	1
11:00	30	4
11:15	30	4
11:30	29	3
11:45	30	4
12:00	31	3
BC-1	18-Mar-97	
time	temp (°C)	#chucks
8:00	12	
8:15	12	
8:30	13	
8:45	14	
9:00	16	1
9:15	16	1
9:30	16	1
9:45	20	2
10:00	22	
10:15	22	2
10:30	22	2
10:45	23	2
11:00	24	
11:15	25	1
11:30	27	1
11:45	27	
12:00	27	1

Table 1 (continued)

BC-1 time	19-Mar-97 temp (°C)	#chucks
8:00	16	
8:15	15	
8:30	18	
8:45	20	
9:00	20	
9:15	21	
9:30	22	
9:45	23	
10:00	24	
10:15	25	
10:30	27	
10:45	26	
11:00	27	
11:15	28	
11:30	28	
11:45	29	1
12:00	28	1

BC-1 time	20-Mar-97 temp (°C)	#chucks
8:00	11	
8:15	17	1
8:30	18	1
8:45	18	1
9:00	19	1
9:15	23	1
9:30	22	1
9:45	23	1
10:00	22	
10:15	26	
10:30	25	
10:45	26	
11:00	25	
11:15	26	
11:30	27	
11:45	28	
12:00	29	

Table 1 (continued)

1999	NB-1 time	21-Mar-99 temp (°C)	#chucks
	8:00	17	
	8:15	20	
	8:30	19	
	8:45	20	
	9:00	21	
	9:15	23	
	9:30	23	
	9:45	23	
	10:00	26	
	10:15	25	
	10:30	26	
	10:45	26	
	11:00	26	1
	11:15	26	
	11:30	25	2
	11:45	26	1
	12:00	26	2
	NB-1 time	22-Mar-99 temp (°C)	#chucks
	8:00	18	
	8:15	22	
	8:30	22	
	8:45	22	
	9:00	22	
	9:15	22	
	9:30	23	
	9:45	22	
	10:00	22	
	10:15	22	
	10:30	22	
	10:45	22	
	11:00	22	1
	11:15	24	1
	11:30	24	2
	11:45	24	2
	12:00	26	1

Table 1 (continued)

NB-1	24-Mar-99	
time	temp (°C)	#chucks
8:00	21	
8:15	20	
8:30	20	
8:45	21	
9:00	21	
9:15	21	
9:30	22	
9:45	22	
10:00	23	2
10:15	23	1
10:30	24	1
10:45	24	2
11:00	24	2
11:15	24	1
11:30	24	2
11:45	25	3
12:00	26	2
V-3	25-Mar-99	
time	temp (°C)	#chucks
8:00	17	
8:15	16	
8:30	18	
8:45	18	
9:00	17	
9:15	18	
9:30	19	
9:45	19	1
10:00	21	
10:15	21	
10:30	20	
10:45	22	
11:00	22	
11:15	20	
11:30	21	
11:45	22	
12:00	22	

Table 1 (continued)

V-3 time	26-Mar-99 temp (°C)	#chucks
8:00	20	1
8:15	23	1
8:30	25	2
8:45	24	3
9:00	23	3
9:15	22	4
9:30	26	4
9:45	23	4
10:00	25	5
10:15	25	4
10:30	25	3
10:45	26	2
11:00	27	5
11:15	27	5
11:30	27	6
11:45	26	1
12:00	27	2
V-3 time	16-Apr-99 temp (°C)	#chucks
8:00	17	
8:15	18	
8:30	17	
8:45	19	
9:00	19	1
9:15	20	1
9:30	21	2
9:45	21	2
10:00	23	3
10:15	23	3
10:30	22	2
10:45	24	5
11:00	24	6
11:15	25	5
11:30	23	5
11:45	24	5
12:00	26	5

Table 1 (continued)

BC-2 time	27-Mar-99 temp (°C)	#chucks
8:00	18	
8:15	19	
8:30	19	
8:45	20	
9:00	22	
9:15	22	
9:30	23	1
9:45	23	1
10:00	24	
10:15	25	2
10:30	26	2
10:45	26	1
11:00	26	2
11:15	27	1
11:30	26	
11:45	27	2
12:00	26	2

BC-2 time	29-Mar-99 temp (°C)	#chucks
8:00	20	
8:15	20	
8:30	21	
8:45	22	
9:00	23	
9:15	24	
9:30	24	
9:45	25	
10:00	26	2
10:15	26	2
10:30	26	1
10:45	27	1
11:00	28	1
11:15	28	1
11:30	30	1
11:45	29	
12:00	29	

Table 1 (continued)

	BC-2	17-Apr-99	
	time	temp (°C)	#chucks
	8:00	16	
	8:15	17	
	8:30	18	
	8:45	22	
	9:00	23	
	9:15	26	
	9:30	26	1
	9:45	27	1
	10:00	27	2
	10:15	27	2
	10:30	27	2
	10:45	27	2
	11:00	28	2
	11:15	28	
	11:30	28	3
	11:45	29	
	12:00	29	1
2000	NB-2	5-Apr-00	
	time	temp (°C)	#chucks
	8:00	24	
	8:15	25	
	8:30	25	
	8:45	25	
	9:00	26	2
	9:15	27	3
	9:30	26	2
	9:45	29	1
	10:00	28	1
	10:15	29	3
	10:30	28	7
	10:45	29	8
	11:00	29	7
	11:15	31	5
	11:30	31	5
	11:45	31	7
	12:00	31	4

Table 1 (continued)

NB-2 time	6-Apr-00 temp (°C)	#chucks
8:00	24	1
8:15	24	1
8:30	24	2
8:45	25	3
9:00	25	3
9:15	26	3
9:30	27	2
9:45	27	2
10:00	28	2
10:15	29	2
10:30	28	3
10:45	27	2
11:00	28	3
11:15	28	2
11:30	28	2
11:45	28	4
12:00	28	4

NB-2 time	7-Apr-00 temp (°C)	#chucks
8:00	23	1
8:15	23	2
8:30	25	3
8:45	27	3
9:00	26	3
9:15	27	4
9:30	27	5
9:45	27	6
10:00	28	4
10:15	30	7
10:30	29	3
10:45	29	4
11:00	30	5
11:15	29	3
11:30	29	4
11:45	29	3
12:00	29	3

Table 1 (continued)

BC-3 time	8-Apr-00 temp (°C)	#chucks
8:00	23	1
8:15	25	2
8:30	25	3
8:45	26	6
9:00	26	5
9:15	27	5
9:30	27	6
9:45	29	7
10:00	29	4
10:15	30	3
10:30	29	2
10:45	30	1
11:00	30	
11:15	31	
11:30	30	
11:45	30	
12:00	30	

BC-3 time	9-Apr-00 temp (°C)	#chucks
8:00	20	2
8:15	20	2
8:30	22	3
8:45	22	4
9:00	24	4
9:15	24	4
9:30	25	3
9:45	25	4
10:00	24	4
10:15	25	5
10:30	25	2
10:45	25	3
11:00	26	2
11:15	27	2
11:30	27	1
11:45	29	4
12:00	28	1

Table 1 (continued)

BC-3	10-Apr-00	
time	temp (°C)	#chucks
8:00	19	
8:15	20	1
8:30	20	2
8:45	20	4
9:00	21	4
9:15	21	6
9:30	22	5
9:45	22	4
10:00	23	3
10:15	23	2
10:30	23	
10:45	23	2
11:00	23	3
11:15	24	1
11:30	24	
11:45	25	
12:00	24	
V-2	11-Apr-00	
time	temp (°C)	#chucks
8:00	20	
8:15	20	
8:30	21	
8:45	20	
9:00	20	
9:15	22	
9:30	22	1
9:45	21	2
10:00	22	2
10:15	23	2
10:30	24	2
10:45	24	3
11:00	24	
11:15	24	
11:30	26	
11:45	26	
12:00	26	

Table 1 (continued)

V-2 time	12-Apr-00 temp (°C)	#chucks
8:00	19	1
8:15	19	1
8:30	24	1
8:45	25	1
9:00	25	1
9:15	26	1
9:30	27	1
9:45	27	1
10:00	26	2
10:15	27	2
10:30	28	2
10:45	28	1
11:00	29	1
11:15	29	3
11:30	30	1
11:45	29	
12:00	29	

V-2 time	13-Apr-00 temp (°C)	#chucks
8:00	20	
8:15	22	
8:30	24	1
8:45	25	1
9:00	26	1
9:15	28	1
9:30	28	1
9:45	28	1
10:00	28	2
10:15	29	4
10:30	28	3
10:45	29	3
11:00	30	1
11:15	31	2
11:30	30	1
11:45	31	
12:00	31	

Size and Age Structure of Study Populations

The measurements and sex of the chuckwallas collected at each site are presented in Appendix B. Summary statistics are presented in Table 2. Animals smaller than 100 millimeters (mm) snout-vent-length (SVL) were difficult to sex so were counted as juveniles. Sex ratios (males/females) showed a strong male bias except for NB-2 and V-3. It is possible that males could be easier to catch and therefore this is biasing the sex ratios. However, we are confident through repeated returns to the sites throughout the season that all or most of the chuckwallas have been removed. Berry (1974) also encountered male sex ratio bias in her four year study of chuckwallas in the northern Mojave (ranging from 1.26 to 1.88) and was confident she captured the population she studied. In addition, her mark recapture data also indicated a greater loss of adult females than males. Nagy (1973) found male sex ratio bias in a study of chuckwallas in the northern Mojave as well. Tracy (1999) did not include one study population from Nevada (Colorock Quarry) in his analysis because the sample size of adult females was too small. However, Johnson (1965) found a sex ratio of 1.09 in favor of females. The strong male bias for chuckwallas in this study does not appear to be unusual when compared to other studies. The reasons for this imbalance are unknown.

Table 2. Summary statistics for animals removed and base line observations from each of the nine sites.

Locality	Observed	Removed	Males/Females	% juveniles	Male wt	Male SVL	Female wt	Female SVL
BC-1	1.3	8	3.00	0.00	248	183	308	192
BC-2	1.3	24	1.33	0.13	204	180	121	156
BC-3	4.5	22	3.00	0.00	297	194	192	159
NB-1	2.0	32	1.89	0.19	147	157	131	157
NB-2	4.3	21	0.88	0.05	185	170	126	150
NB-3	3.0	38	1.58	0.18	151	157	86	137
V-1	3.5	51	2.62	0.08	227	189	141	150
V-2	1.7	4	all males	0.00	231	181	NA	NA
V-3	2.7	20	0.67	0.00	261	191	85	130

Animals from the northern populations (Bonnie Claire and Virgin Mountains) appear to be larger than animals from the southern population (Newberry Mountains). Although the nine outcrops were roughly the same size, they varied in complexity. Sites V-1 and NB-3 could be ranked as the "best" habitat within their respective groups. These sites contained a greater amount of smaller outcrops within the greater outcrop. Chuckwallas seem to prefer to bask on these smaller outcrops. They may prefer these outcrops for a number of reasons that have not been tested. The smaller outcrops may provide a basking chuckwalla with a greater field of vision for detecting predators while they bask. They also may provide the greatest exposure to the morning sun. These two outcrops (V-1 and NB-3) also had the greatest number of animals removed within their respective groups.

Relative Abundance Index

Figure 5 shows the relationship between the average median numbers of chuckwallas observed for each site during the survey and the respective numbers of chuckwallas removed. We were unable to recover a meaningful linear relationship for management purposes among the numbers of animals observed and the number of animals removed. The regression equation is 12.2 + 4.5 (*observed*) = *removed* ($p = 0.35$; $t_{(1,8)} = 1.1$; R-Square=0.15). The numbers of animals we observed at each site were not reliable predictors of how many animals could be removed from the site. Previous studies on chuckwallas also expressed difficulty in estimating population numbers.

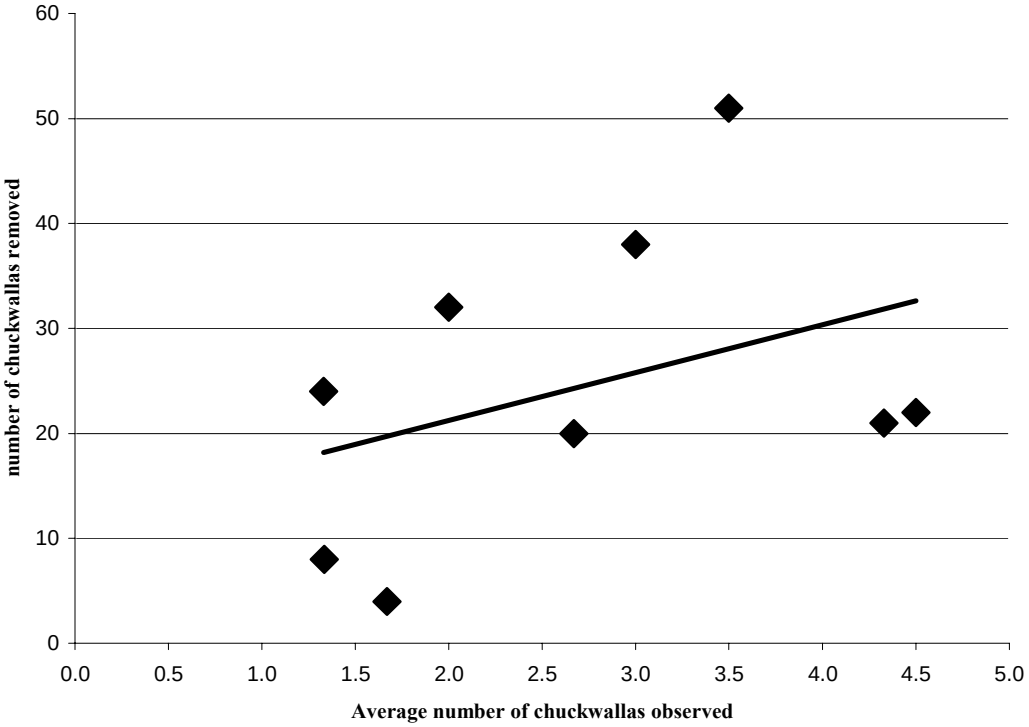


Figure 5. Dot plot distribution showing the relationship between the average median numbers of chuckwallas observed for each site during the survey and the respective numbers of chuckwallas removed. The regression equation for the line is 12.2 + 4.5 (*observed*) = *removed* ($p = 0.35$; $t_{(1,8)} = 1.1$; R-Square=0.15).

It seems for future census purposes that there is a window of time when most of the animals are out in easy to see basking sites, yet are not moving so they can be more easily counted. This window is from April to May at around 10:00 AM between air temperatures of 81°F and 86°F (28°C and 30°C). At best, the most reliable method of monitoring chuckwalla populations are presence/absence surveys by actually observing lizards or finding fecal pellets on rocks based on what was found in monitoring repatriation of removed populations.

MEDIATED POPULATION DISTURBANCE

We took the opportunity to monitor recovery rates in the experimentally removed populations to access recovery rates in populations that have experienced a significant decline in numbers. In the successive seasons following removal, the sites were surveyed for chuckwallas by spotting scope and by searching cleaned basking sites for new fecal pellets to monitor recovery rates.

Repatriation observations are presented in Table 3. All but one (BC-1) of the populations removed experienced a decrease in the number of chuckwallas observed in subsequent seasons relative to pre-removal observations. In addition, the fecal pellets piles showed little to no use compared to pre-removal quantities. The removal study implied that chuckwalla populations so affected may be slow to return to pre-removal observations. This is consistent with what is known about chuckwalla biology. Chuckwallas are a relatively long lived lizard. Females have small clutches of proportionally large eggs, experience little or no detectable migration, and are habitat specialists. All of these factors contribute to an organism that one would suspect is susceptible to slow recovery rates after experiencing a population crash.

Table 3. Observations of chuckwallas at each site in subsequent seasons where chuckwallas were removed in 1997, 1999, and 2000.

Site	Year	Month	Time	Temp. (˚C)	Males	Females	juveniles	Total
V-1	1998	April	11:30	31	2	0	0	2
		May	10:30	33	1	0	0	1
		June	9:30	32	0	0	0	0
	1999	April	11:45	25	0	0	0	0
		May	10:30	25	0	0	0	0
		June	8:15	29	0	0	0	0
	2000	April	10:00	30	1	0	0	1
		May	9:00	31	0	0	0	0
		June	10:30	32	0	0	0	0
	2001	April	9:00	25	1	1	0	2
		May	10:00	28	0	1	0	1
		June	10:15	30	0	1	0	1
NB-3	1998	April	11:00	31	0	0	2	2
		May	10:30	32	0	0	0	0
		June	10:30	31	1	0	0	1
	1999	April	11:15	30	0	0	0	0
		May	10:00	30	0	0	2	2
		June	9:00	29	0	0	1	1
	2000	April	11:15	30	0	0	0	0
		May	10:30	30	0	0	0	0
		June	9:30	29	0	0	0	0

Table 3 (continued)

Site	Year	Month	Time	Temp. (˚C)	Males	Females	juveniles	Total
NB-3	2001	April	8:15	30	0	2	0	2
		May	12:00	32	0	0	0	0
		June	8:30	30	0	0	0	0
BC-1	1998	April	10:45	22	1	1	0	2
		May	10:30	25	0	1	0	1
		June	10:30	26	0	0	2	2
	1999	April	12:00	23	0	0	0	0
		May	10:15	26	1	1	0	2
		June	11:00	28	0	0	0	0
	2000	April	12:00	24	0	0	0	0
		May	10:00	28	0	0	0	0
		June	10:00	32	0	0	0	0
	2001	April	10:00	25	2	1	1	4
		May	9:00	24	0	0	0	0
		June	10:00	28	1	1	0	2
NB-1	2000	April	9:30	31	0	0	0	0
		May	10:00	25	0	0	0	0
		June	11:00	30	0	0	0	0
	2001	April	11:00	32	2	0	0	2
		May	10:00	29	1	0	0	1
		June	12:00	31	0	0	0	0
V-3	2000	April	11:15	26	0	0	0	0
		May	10:00	32	0	0	0	0
		June	9:45	29	0	0	0	0
	2001	April	11:30	28	1	1	0	2
		May	10:00	30	0	1	0	1
		June	8:15	32	0	0	0	0
BC-2	2000	April	12:00	28	0	1	0	1
		May	10:15	29	0	0	0	0
		June	10:00	27	0	0	0	0
	2001	April	11:00	27	0	0	0	0
		May	11:30	30	1	0	0	0
		June	11:45	32	1	0	0	1
V-2	2001	April	11:00	26	0	0	0	0
		May	11:30	28	0	0	0	0
		June	8:45	33	0	0	0	0
NB-2	2001	April	9:45	29	2	0	0	2
		May	10:30	28	0	0	0	0
		June	8:00	27	0	0	0	0
BC-3	2001	April	10:00	26	0	0	0	0

Table 3 (continued)

Site	Year	Month	Time	Temp. (˚C)	Males	Females	juveniles	Total
		May	12:00	31	1	0	0	0
		June	12:00	32	1	0	0	1

GENETIC STRUCTURE OF CHUCKWALLA POPULATIONS IN NEVADA

Why Study Genes?

Knowledge of the genetics of species of special concern is now the basis of an important and critical area of species management. The potential for populations to adapt to environmental pressures is proportional to the amount of genetic diversity available (Fisher, 1958). When genetic diversity is low, the rate and scope of the population's response to new environmental conditions is reduced. Genetic diversity within a species exists at three levels: variation within individuals, among individuals within a population, and differences among populations. Each level is a genetic resource of potential importance to conservation, so each must be understood relative to the others (Meffe and Carroll, 1994). A genetic based investigation of a species population structure may provide an opportunity to predict the organism's response to environmental pressures. Therefore, it is critical to identify the levels of genetic variation exhibited in chuckwallas within Nevada in order to delimit potential units for more effective conservation and management.

Known Levels of Genetic Variation in Chuckwallas

Geographic Variation

The chuckwalla over its entire range is presently considered to be a single species that displays geographic variation in color pattern and scale counts (Hollingsworth, 1998), and life history (Case, 1976; Tracy, 1999). In addition, previous studies on chuckwallas reveal an animal with some of the greatest level of genetic variation in any animal surveyed. Out of 51 chuckwallas assayed across the species' distribution, Lamb, Jones, and Avise (1992) found 30 unique mtDNA haplotypes (a haplotype is a distinct genetic marker). Petren and Case (1997) found 27 unique mtDNA haplotypes out of 32 chuckwallas assayed. These studies indicate that chuckwallas possess a great amount of genetic variation throughout their range. However, they hesitated to draw any conclusions about intra and inter population diversity due to small sample sizes per locale (Lamb et al., 1992) or limited sampling from extremely diverse populations as intermediate haplotypes may exist in unsampled geographic areas (Petren and Case, 1997).

Population Variation

Tracy (1999) found variation among populations at a smaller geographic scale. Using common garden experiments, he surmised genetic differentiation among populations in close geographic approximation. The populations he studied showed evidence of having differences particular to the environmental conditions in which they lived, particularly rainfall and elevation. Kwiatkowski and Sullivan (2002*a*) documented variation in female mate choice among populations in three mountain ranges in the Phoenix, Arizona area. The choices females made were demonstrated to be based on physical differences among males particular to the population examined.

Chuckwallas possess numerous derived morphological and behavioral characteristics associated with seeking refuge within rock crevices. Rock outcrops containing suitable habitat are found within the mountain ranges that are surrounded by broad valleys and basins with no suitable chuckwalla habitat. As such, suitable habitat is patchy and often isolated. Chuckwallas exhibit several life-history characteristics that should accentuate limited opportunity for genetic exchange between populations. Life history characteristics such as delayed maturity, high adult survivorship, low annual reproductive frequency, and limited migration (confined mostly to males) are traits that may be conducive to population differentiation in chuckwallas (Johnson, 1965; Berry, 1974; Abts, 1987). Given chuckwallas highly adapted biology to living on rocks and the disjunct distribution of rock outcrops suitable for habitation, one may expect this may lead to low levels of migration and gene movement between populations.

Nevada Populations

Distinct differences in male dorsal and ventral color patterns among mountain ranges in Nevada exist, implying genetic differentiation (Figure 6). Three distinct color pattern types exist. Hollingsworth's (1998) Northern Speckled color pattern dominates the north western part of their range in Nevada and the Virgin River color pattern prevails in the mountains east of the Las Vegas Wash. Color pattern of chuckwallas in the southern most part of Nevada (in the Newberry Mountains) more closely resembles the Partially Melanistic Chuckwalla. On closer examination, color patterns in the northern are merely a variation on the same basic striped dorsal color pattern. Some populations are darker than others with faint hints of stripes, while others are more obvious. Our observations on color pattern at this small scale agree with what Hollingsworth (1998) found over a broader geographic range.

Different environmental conditions among geographically distinct populations have been shown in previous studies to influence differences in traits such as color pattern, growth rates, and mating strategies (Tracy, 1999; Kwiatkowski and Sullivan, 2002*a*). This suggests that chuckwalla populations on different mountain ranges have the potential to be on different evolutionary trajectories. Therefore, it is important to characterize any distinctions among chuckwalla populations in Nevada in order to delimit potential units for more effective conservation and management.

Determining Genetic Structure

Population subdivision is often caused by environmental patchiness, areas of favorable habitat intermixed with unfavorable areas. Patterns of mtDNA phylogeographic structure appear to be influenced by differences in organisms' suspected migration abilities and in environmental fragmentation (Avise, 2000). Organisms whose biology and habitat requirements suggest low levels of gene flow exhibit pronounced spatial-genetic structure such as pocket gophers (genus *Thomomys*) (Patton and Smith, 1989; Smith, 1998), pond breeding salamanders (genus *Ambystoma*) (Phillips, 1994; Templeton et al., 1995; Shaffer and McKnight, 1996), flightless brown kiwi (*Apteryx australis*) (Baker et al., 1995), and freshwater fishes across isolated drainages (Avise et al., 1984; Bermingham and Avise, 1986). Because of their suspected low levels of gene flow and strict habitat requirements, chuckwallas should make suitable candidates for such study.

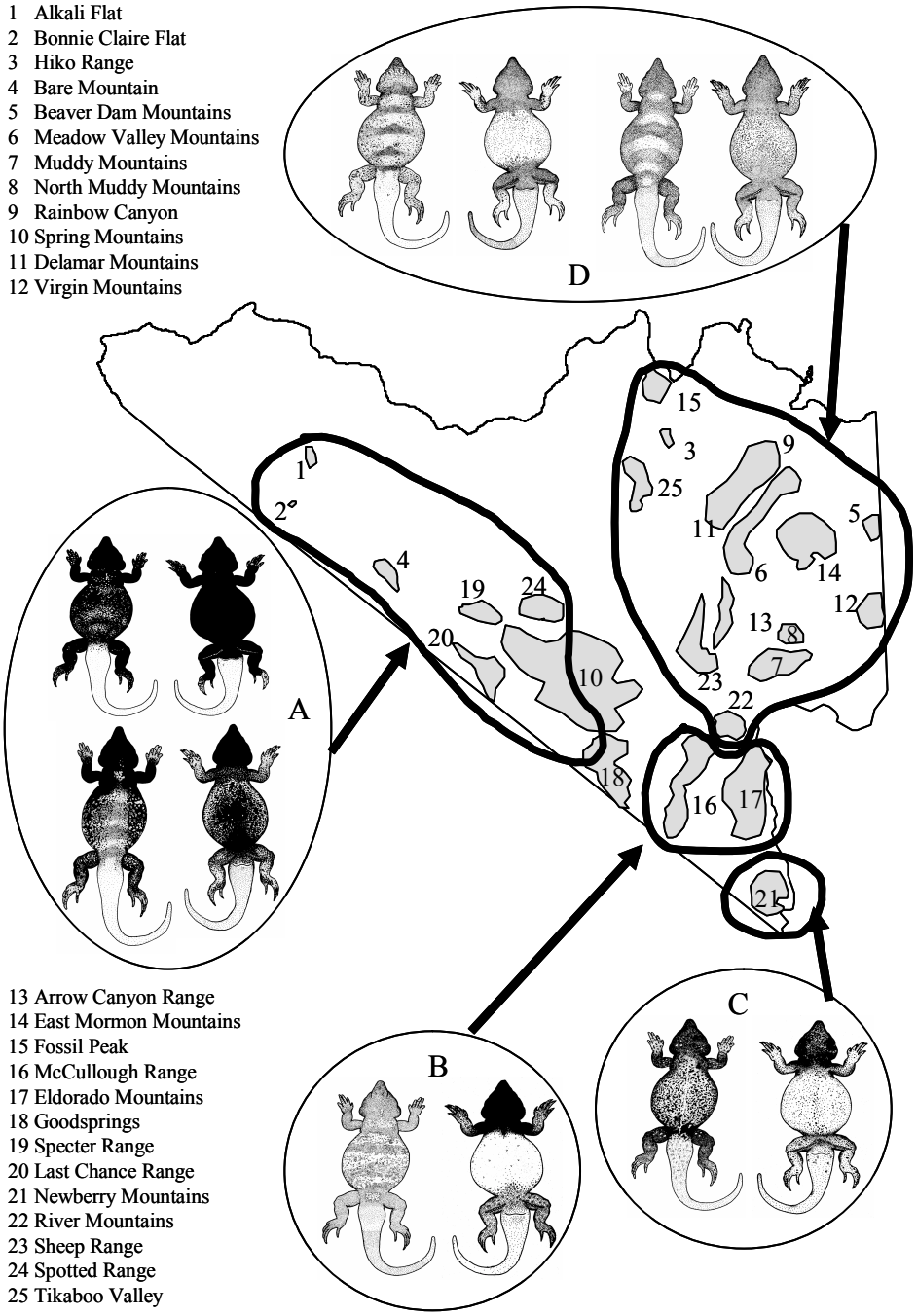


Figure 6. Dorsal (left) and ventral (right) color pattern variation for chuckwallas (*Sauromalus obesus*) in the Nevada Mojave Desert ecoregion surveyed in this study. A) Northern Speckled; B) "Eldorado" Virgin River; C) Newberry Mountains; D) Virgin River

One method of recovering processes in such data is the phylogeographic method. Phylogeography involves the principles and processes that govern the geographical distribution of genealogical lineages as defined by Avise and his co-workers over a decade ago (Avise et al., 1987) and most recently emphasized in Riddle's (1996) review. Two types of patterns must be recovered: 1) current geographical distribution of populations of the species in question and 2) a reliable genealogical lineage among the populations. Once molecular data are analyzed using phylogenetic methods, the resulting phylogeny is overlaid on the current geographic distribution and patterns are interpreted in light of the animal's biology and past climatic or geologic events. We characterize the current geographic distribution of chuckwallas among selected mountain ranges by means of a predictive habitat relations model using a Geographic Information System (GIS) developed and presented in the 1995 report and field tested in this report. We use the isolated populations predicted in the model as sample units for the phylogenetic analysis of mitochondrial DNA (mtDNA) haplotypes. We test the interpretation of the phylogeographic analysis with a little used analytical method pioneered by Templeton and his co-workers (Templeton et al., 1995). This analysis involves an overlay of geography on an estimated gene tree in a rigorous statistical framework designed to measure the strength of any geography/phylogeny associations. All of this is discussed in light of the biology of chuckwallas and paleoecological evidence for recent climatic and vegetation changes in this region.

We characterized the patchy nature of suitable chuckwalla habitat among mountain ranges with the GIS model. The model suggested suitable habitat was clustered within mountain ranges. These mountain ranges are separated by unsuitable habitat. Based on the model, we sampled chuckwallas from mountain ranges within the Mojave Ecoregion in Nevada for genetic analysis. The following mountain ranges were subjectively identified based on the model as populations to be sampled in the genetic analysis: Newberry Mts., Eldorado Mts., McCullough Mts., River Mts., Spring Mts., Goodsprings ("southern" Spring Mts.), Last Chance Range, Spotted Range, Specter Range, Bare Mt., Bonnie Claire, Stonewall Mts., Sheep Range, Arrow Range, Muddy Mts., North Muddy Mts., Virgin Mts., Tule Hills, Mormon Mts., Delamar Mts., Meadow Mts., Hiko Range, and the Mt. Irish Range. Mountain ranges were visited in 1998 and an attempt was made to collect five tissue samples from each range, given the extensive mtDNA polymorphism found in chuckwallas from previous studies. Given their highly restrictive habitat requirements and the patchy distribution of such suitable habitat, we expect the phylogenies we recover to exhibit a high degree of genetic population structure among mountain ranges.

Specimen Information

Sampling sites in southern Nevada are shown in Figure 7. Mitochondrial DNA sequences were examined from 105 individuals, which included 104 ingroup specimens from 25 neighboring mountain ranges determined from the habitat model in chapter one, one outgroup individual from a non neighboring mountain range approximately 270 kilometers south of the nearest ingroup mountain range, and one outgroup sequence obtained from (GenBank accession number AF020232) Carbaca, Sonora, Mexico. Because some ingroup specimens were identical for the regions of the genes sequenced,

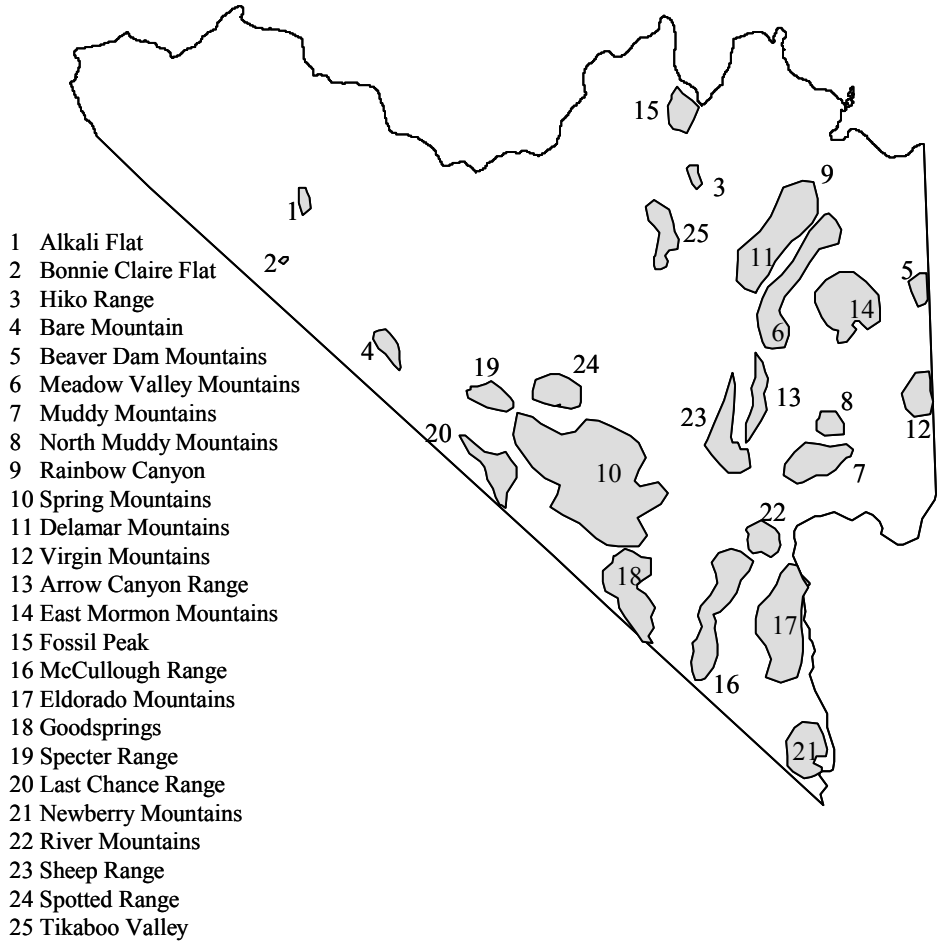


Figure 7. Mountain ranges in Nevada in which Chuckwallas (*Sauromalus obesus*) were sampled for the genetic analysis.

one sequence for each unique haplotype from every locality sampled was used in the phylogenetic analysis. Thus, the final data set includes only unique haplotypes from all the localities sampled. Voucher specimens for this study are deposited at The University of Texas at Arlington (UTA). Voucher numbers and locality information for each specimen are listed in Appendix C. Haplotype labels of each unique haplotype for each gene fragment are listed in Appendix D. Sequences of the unique haplotype fragments will eventually be accessioned in GenBank pending publication in a peer reviewed journal.

Laboratory Protocols and Analysis of Data

We collected tissue samples in the field, extracted DNA from the tissues in the lab, and then isolated fragments of mtDNA for each individual: one from the cytochrome *b* region on the mtDNA and the other from the control region. We then identified the order of the sequence of nucleotide bases for each fragment (haplotype) and compared them among individuals and among populations. We interpreted the variation in terms of their evolutionary relationships using analysis techniques known as Parsimony Analysis, Maximum Likelihood Analysis, and TCS. These techniques are different ways of interpreting the genetic relationships graphically among the sequences in the form of branching trees and networks. These representative trees are referred to as phylogenies. Technical details of laboratory protocols, raw data, and analysis are found in Appendices E, F, and G. What follows is a summary of those findings.

Mitochondrial DNA variation in Nevada Chuckwallas

Variation among individuals within a population

Variation for cytochrome *b* among individuals within a population was very low, if any was present at all. If it did vary, differences in the sequence between individuals within a population were never more than one nucleotide base pair (bp). Only one population (Newberry Mountains) exhibited variation greater than one bp among individuals (three bp). Variation for the control region among individuals within a population was very low, if any was present at all. Differences between individuals ranged from one bp for several populations and three bp for individuals within the Newberry population only.

Variation among populations

The greatest bp distance between individuals for the cytochrome *b* haplotype was 24, between haplotypes T and A and T and K. Haplotypes T and A came from the two populations that also had the greatest geographic distance between them, 336 kilometers (Newberry Mountains and Stonewall Mountain). However, haplotypes T and K (Newberry Mountains and Goodsprings) are closer in geographic distance to each other (106 km). Of the 25 mountain ranges surveyed 15 ranges had at least one unique cytochrome *b* haplotype (Hiko Range, Bare Mountain, Beaver Dam Mountains, Eldorado Mountains, Mt. Irish, Specter Range, Goodsprings, Spring Mountains, McCullough Mountains, Meadow Valley Mountains, Newberry Mountains, River Mountains, Sheep Range, and Spotted Range). One haplotype (D) was found in 37 individuals distributed among a centrally located cluster of 11 adjacent mountain ranges (Meadow Valley

Mountains, Muddy Mountains, North Muddy Mountains, Rainbow Canyon, Spring Mountains, Arrow Canyon Range, Delamar Mountains, River Mountains, Virgin Mountains, East Mormon Mountains, and Mt. Irish Range). The rest of the haplotypes found in more than one mountain range were more or less between adjacent mountain ranges. Haplotype A was found in Stonewall Mountain and Bonnie Claire Flat. Haplotype E was found in the Eldorado Mountains and the McCullough Mountains. Haplotype L was found in the Newberry Mountains and Goodsprings. Haplotype N was found in the Specter Range and the Last Chance Range. Haplotype W was found in the Specter Range and the Spotted Range.

The greatest difference between individuals for the control region haplotype (25 bp), was between haplotypes (AB and BE, Muddy Mountains and Newberry Mountains) but these were not localities separated by the greatest geographical distance. All of the 25 mountain ranges sampled contained at least one unique control region haplotype. Only two haplotypes (AN and AM) were shared among more than one mountain range. Haplotype AN was found in 6 individuals distributed among a centrally located cluster of 4 mountain ranges (Spring Mountains, Arrow Canyon Range, Meadow Valley Mountains, Delamar Mountains, River Mountains). Haplotype AM was represented from five of the six individuals sampled in the Last Chance Range and from one of six sampled from the Specter Range.

Genetic relationships

Our analyses supported two distinct genealogical lineages (clades) of chuckwallas among the mountain ranges sampled in southern Nevada. One clade consists of haplotypes from the Newberry Mountains and Goodsprings. The other clade includes haplotypes from all the other populations north of the Newberry Mountains. A more detailed analysis among the populations within the two distinct clades suggests that chuckwalla populations among mountain ranges are currently experiencing very little or no gene flow.

Maximum parsimony analysis of the cytochrome *b* data set produced 49 trees that are all equally possible to represent the genetic relationships. A more conservative analysis (using the bootstrap method) retaining only those branches with ≥50% statistical support two clades: haplotypes from the southern most mountain ranges (Newberry Mountains and Goodsprings) and all the other northern mountain ranges (Figure 8A). Phylogenetic structure within each clade was minimal. Only two internal clades were supported within the northern populations. Haplotypes F and G (one bp difference) sampled from the same population (Eldorado Mountains) formed an internal clade. The internal clade containing haplotypes M and X represent populations from adjacent mountain ranges (Spotted Range and Spring Mountains) and separated by 22 km. The southern clade contains only two populations (Newberry Mountains and Goodsprings) but contains five haplotypes. These formed two internal clades with no respect to population and haplotypes differing by three bp.

Maximum parsimony analysis of the control region data set produced $>10^6$ trees. Bootstrap analysis retaining only those branches with ≥50% support revealed the same two clades recovered from the cytochrome *b* data set (Figure 8B) and even less within clade structure. Only two clades were supported within the northern clade. Haplotypes I, J, and K (one to three bp differences) sampled from the same population (Eldorado Mountains) formed an internal clade. The internal clade containing haplotypes A and F

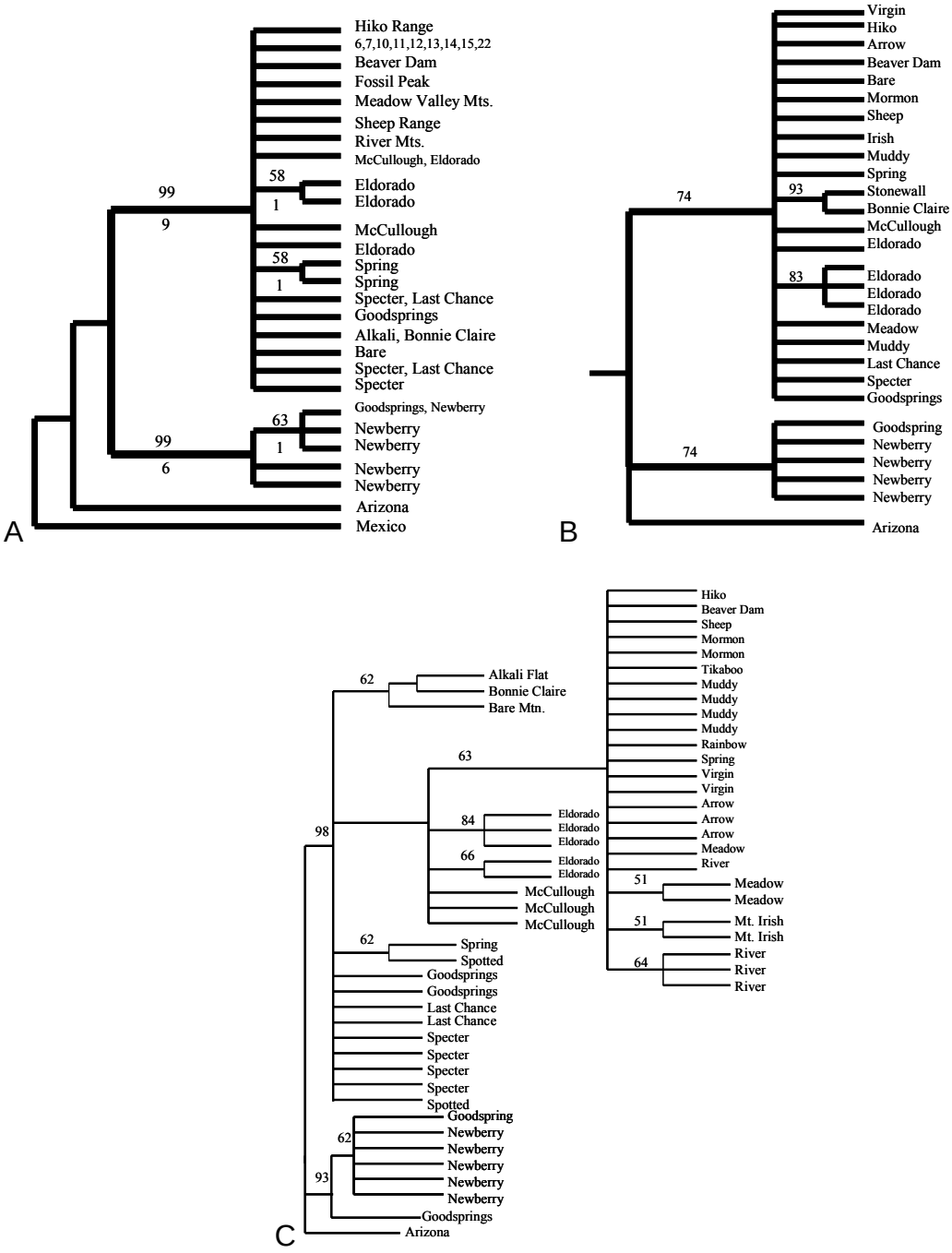


Figure 8. Consensus trees resulting from the maximum parsimony analysis of the A) cytochrome *b* fragment haplotypes (49 trees), B) control region fragment haplotypes (>10^6 trees), and C) combined cytochrome *b* and control region fragment haplotypes (>10^6 trees). Locality numbers at tips pertain to Figure 7. Numbers above branches are branches with greater than 50% bootstrap support. Numbers below are decay indices (Bremer, 1994).

(Stonewall Mountain and Bonnie Claire Flat, one bp difference) were from the two most geographically distance and isolated populations relative to the rest of the populations sampled. The southern clade contains only two populations (Newberry Mountains and Goodsprings) but contains eight haplotypes. Although the range of bp differences among them ranged from one to eleven, there was no phylogenetic resolution.

Maximum parsimony analysis of both data sets combined produced $>10^6$ trees. Bootstrap analysis retaining only those branches with ≥50% bootstrap support revealed the same two clades recovered from the cytochrome *b* data set and the control region data set (Figure 8C). However, phylogenetic structure within each clade was greater than either data set could reveal alone but provides no more resolution. Haplotypes from the same populations tended to clade together at the tips of the topology but offer no deeper resolution within each clade.

Maximum likelihood for the cytochrome *b* data set revealed the same two north and south clades that were recovered from the maximum parsimony analysis (Figure 9A). Substructuring was similar with a few exceptions. In the northern clade, the F and G clade was retained with greater bootstrap support (80 as opposed to 53 for cytochrome *b*) and the M and X clade was retained but nested within a clade containing B (Bare Mountain) and W (Spotted Range and Specter Range).

Maximum likelihood for the control region data set revealed the same two north and south clades, both with bootstrap support (Figure 9B). However, there was more substructuring within these two clades, especially within the northern clade. This northern clade was split into two clades: the "black clade" and the "striped" clade. These clades separate geographically and by male color pattern. The black clade is comprised of haplotypes from a group of mountain ranges clustered in the northwest region within their range in Nevada (Stonewall Mountain, Bonnie Claire Flat, Bare Mountain, Specter Range, Spring Mountains, and the Last Chance Range). The adult males from these populations have a predominately black color pattern on both the dorsal and ventral surfaces of the body (Figure 10). The haplotype in this clade from Goodsprings (P) was assayed from one female and one juvenile male so it remains to be seen if the specific haplotypes agree with adult male color pattern in this population. The striped clade is comprised of haplotypes from all the remaining mountain ranges above the Newberry Mountains. The adult males from these populations have a predominately striped dorsal color pattern and a cream ventral pattern (Figure 10). In addition, color patterns among adult males in the Newberry Mountains are different from the northern clade (Figure 10).

Although more structure was recovered with maximum likelihood, branch lengths on the tree were short. Short branch lengths reflect the overall low level of mtDNA divergence between these populations. In addition, there was little bootstrap support for the internal clades. Even though each mountain range sampled had unique haplotypes for chuckwallas assayed for the control region fragment, there was not sufficient difference to detect any deeper historical relationship among populations within the two clades.

We detected two genetically distinct clades of chuckwallas among the mountain ranges sampled in southern Nevada. One clade consists of haplotypes from the Newberry Mountains and Goodsprings. The other clade includes haplotypes from all the other populations north of the Newberry Mountains. While the analyses recovered strong support for the distinctness of the two clades, intraclade affinities were more ambiguous. Parsimony analyses did not reliably support any bifurcating topology among them

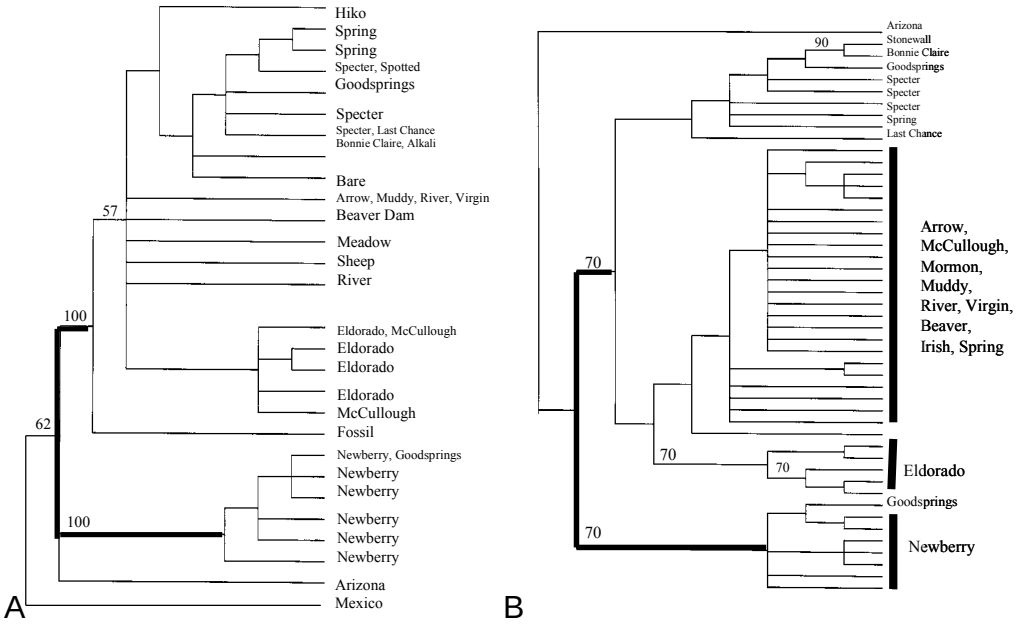


Figure 9. Maximum likelihood analysis of A) cytochrome *b* fragment haplotypes (one tree), and B) control region fragment haplotypes (one tree). Numbers on branches are branches with greater than 50% bootstrap support.

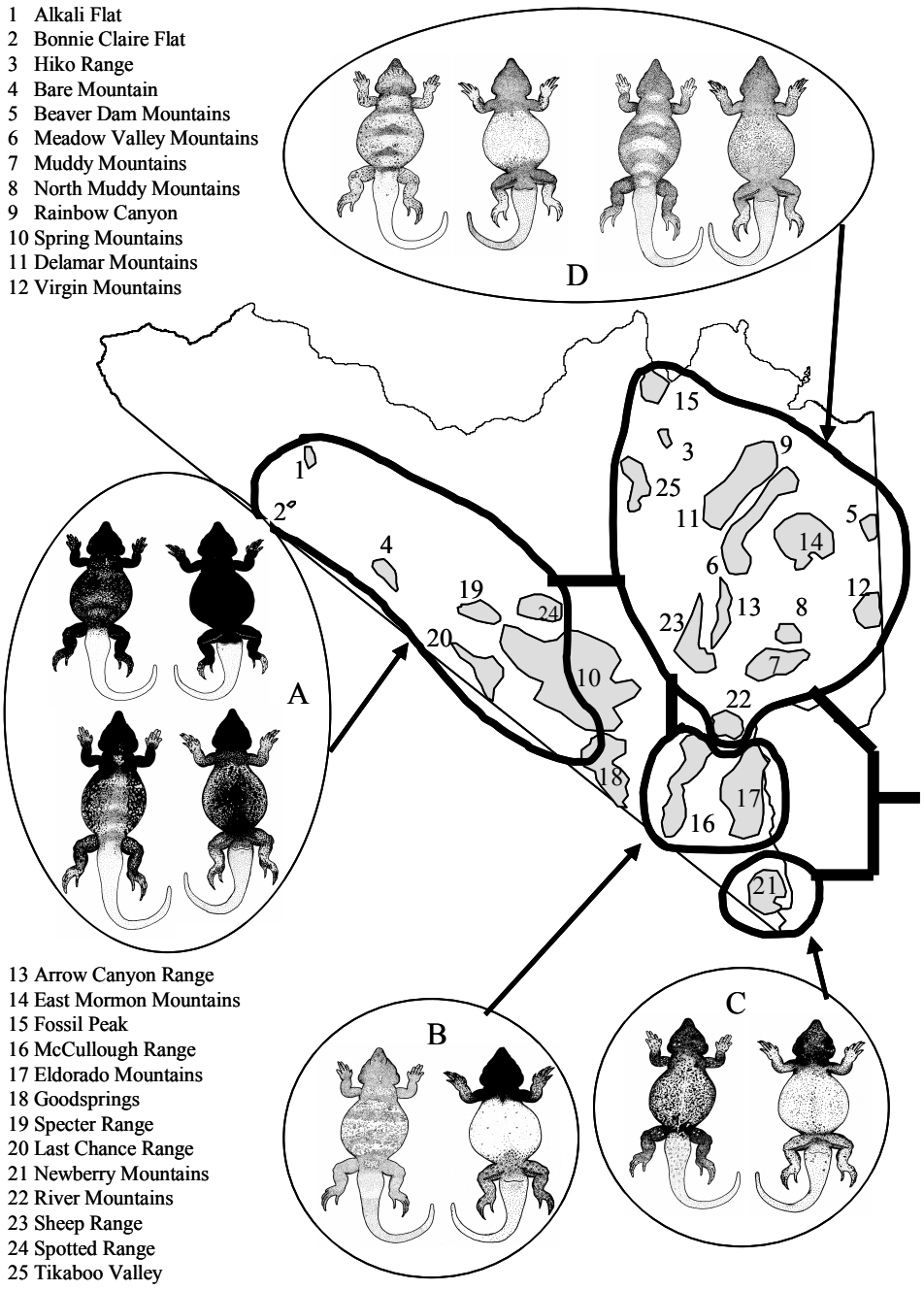


Figure 10. Phylogeographic dorsal (left) and ventral (right) color pattern variation for chuckwallas (*Sauromalus obesus*) in the Nevada Mojave Desert ecoregion, noting the two clades with strong support from maximum parsimony and maximum likelihood analyses of mtDNA. A) Northern Speckled; B) "Eldorado" striped; C) Newberry Mountains; D) Virgin River striped.

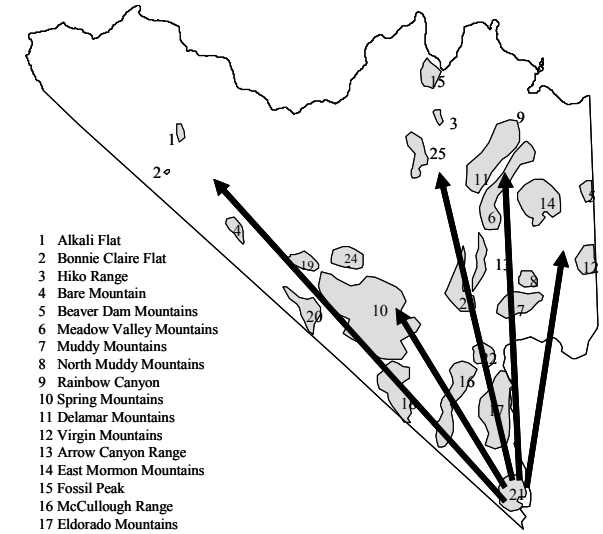
(Figure 8). Maximum likelihood suggested greater partitioning within the northern clade but with little bootstrap support. These results suggest one or all of three mechanisms causing a lack of intraclade resolution: (1) recent colonization, (2) present day continuing gene flow, and (3) lack of power to estimate an accurate phylogeny.

The best explanation of low genetic divergences and the interpretation of the biogeographic signature of the genetic patterns among populations of chuckwallas in southern Nevada is most likely dominated by historical forces. We believe that a combination of genetic fixation and subsequent relatively recent colonization best accounts for the results. This conclusion is grounded in the following observation: much of the current habitat occupied by chuckwallas in southern Nevada was unsuitable chuckwalla habitat (cold desert) during the last glacial period in the Pleistocene (Spaulding, 1990*a,b*). Consequently, chuckwallas have colonized the majority of the mountain ranges in Nevada above the Newberry Mountains from southern warm desert sources within the last ~10,000 years.

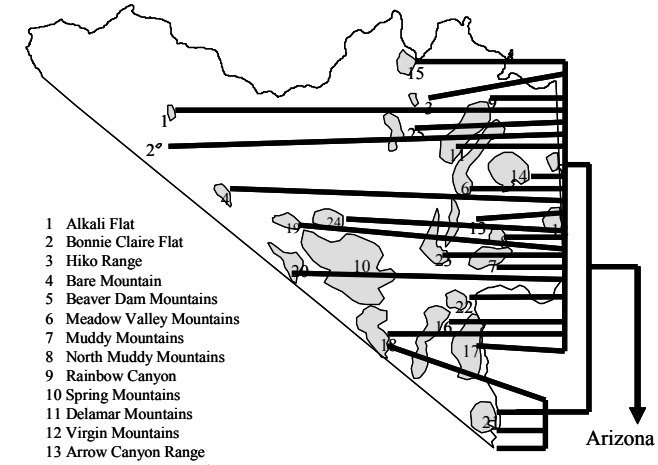
All the analyses are most in accord with the hypothesis that chuckwallas occupied the northern most extension of warm desert habitat up the Colorado River trough (Newberry Mountains) during the Wisconsin glacial period of the Pleistocene (Holman, 1995). Fixation of mtDNA variants in the Newberry Mountain population occurred over this extended period of time, followed by colonization of previously unsuitable habitat as the present warm desert climate moved north (Holman, 1995; Hockett, 2000)(Figure 11). Once chuckwalla pioneer populations persisted at previously uninhabited suitable habitat, movement among mountain ranges was rare because of their specific habitat preferences and life history characteristics such as delayed maturity, high adult survivorship, low annual reproductive frequency, and limited migration (confined mostly to males) that may be conducive population differentiation in chuckwallas (Johnson, 1965; Berry, 1974; Abts, 1987). This is exemplified by the high numbers of haplotypes unique to mountain ranges. However, since they are recent arrivals, there has not been enough time for these populations to diverge enough to be detected in a phylogenetic analysis using these gene fragments. The occurrence of a shared haplotype among populations concentrated along the White River drainage is consistent with a pattern that suggests that early migrants dispersed from this drainage into the surrounding areas.

Current evidence for assembling the vegetation communities during the full glaciations of the Southwest comes from pollen analysis and packrat (genus *Neotoma*) midden analysis. Martin and Mehringer (1965) synthesized their own work and previous studies to make a map of Wisconsin age vegetation based on pollen record analysis. In this reconstruction, warm desert vegetation persists to the north along the Colorado River trough. Habitat outside the Colorado River trough is cold desert sagebrush and pinyon juniper woodland, habitat that is unsuitable for chuckwallas today.

More convincing support comes from the analysis of layered mounds of well-preserved fragments of vegetation and bone fossils collected by packrats and often encased, much like insects in amber, in crystallized urine (Betancourt, et al., 1990). These mounds are referred to as middens and are often found in caves and rock crevices throughout the arid southwest. There are six packrat species that occur or have occurred in the southwestern United States for the last forty thousand years (Betancourt, et al., 1990). Packrats that live in arid or semi-arid habitats are unable to withstand the high diurnal temperatures characteristic of the southwestern deserts (Lee, 1963). These rodents rely on behavioral adaptations in the form of midden construction in order to live



A



B

Figure 11. A) Post-Pleistocene chuckwalla (*Sauromalus obesus*) range expansion into the northern Mojave from Sonoran refugia as implied by mtDNA analysis B) Phylogeographic overlay of the most conservative consensus of maximum parsimony and likelihood analyses of cytochrome *b* and control region mtDNA fragments supports a northern Mojave clade and a Newberry Mountain clade.

in areas where the physical conditions often extend beyond their physiological limits. Internal temperatures of middens can be nearly 10˚C below the ambient temperature at the entrance and more humid as well (Lee, 1963). Packrats build these middens in rock piles, crevices, caves, or dense patches of vegetation for resting, sleeping, food storage, and giving birth to young. The middens are composed of a superficial, outer layer of haphazardly arranged sticks, rocks, bones, and shrubbery. The interior is composed largely of a quantity of coherent materials (soil, grass, feces) that the packrat encases into a concrete-like mass with crystallized urine. This interior is perforated by interconnecting passages and chambers. Packrat midden contents can represent a sample of the vegetation gathered by the animal within its home range around the midden (around 402 square meters; Bleich and Schwartz, 1975). The same midden location can be used by generations of packrats over time. The contents of middens located in rock caves or crevices can be remarkably preserved by the preservation properties of the desert (low humidity, extreme temperatures, high evaporation rates, etc). In addition, middens located in rocks represent the unique plant community of the rocky outcrop—the same habitat shared by chuckwallas. As a result, middens can provide evidence towards the construction of the historical ecology of habitat now used by chuckwallas. Incidentally, the shared habitat of chuckwallas and packrats is so intimate that H. C. Yarrow and S. F. Baird, prominent zoologists in their time, thought middens were deposited by chuckwallas (Yarrow, 1875). Paleontologist/herpetologist Edward D. Cope got it right by "believing them to be the excrement of small mammals, such as *Neotoma*" (Yarrow, 1875, pg. 562).

In their extensive synthesis of packrat midden studies, Betancourt, et al (1990) suggest that the distributions of the deserts of the arid southwest shifted radically perhaps with Plio-Pleistocene uplift of the Sierra Nevada and with the waxing and waning of each ice age. From 30,000 to 12,000 years ago, while the glaciers still existed up north, what is mostly now warm desert scrub in southern Nevada was cold, arid scrub land (*Artemisia*, juniper, pinyon juniper, and pinyon-juniper-oak woodland)(Betancourt, et al., 1990). The Colorado River trough supported a warm desert refugia that extended at least as far north as the Newberry Mountains (Figure 12).

Packrats also collect bone laden carnivore feces, parts of prey skeletons discarded by carnivores, or bones of small vertebrates that live in the rocks around or even use packrat dens as shelter (such as chuckwallas). Chuckwalla evidence appears in midden data in this southern region as old as 24,000 years ago (Van Devender and Mead, 1978; Van Devender, et al, 1977). Chuckwalla fossils don't show up in the northern sites outside of the Colorado River trough until around 10,000 years (Norell, 1986; Brattstrom, 1954). These fossils are from individual packrat middens, from cave sites lacking stratiographic control or multiple radiocarbon dates, or open-air contexts. In Pintwater Cave, Hockett (2000) recovered a faunal assemblage from an undisturbed matrix with multiple radiocarbon dates. Pintwater Cave is located in the southern Pintwater Range in the northern Mojave Desert of southern Nevada, well within the northern range of chuckwallas in the present day Mojave Desert. The Pintwater Range (although not sampled in our genetic analysis since it lies within the Nellis Air Force Bombing and Gunnery Range) is among mountain ranges that we sampled that are part of the northern clade. Hockett (2000) recovered over 70,000 bones from a stratified excavation of a pile of degraded owl pellets and carnivore scats. Radiocarbon dates were obtained from six levels excavated, producing a chronologically ordered suite of dates from 7,350–32,000

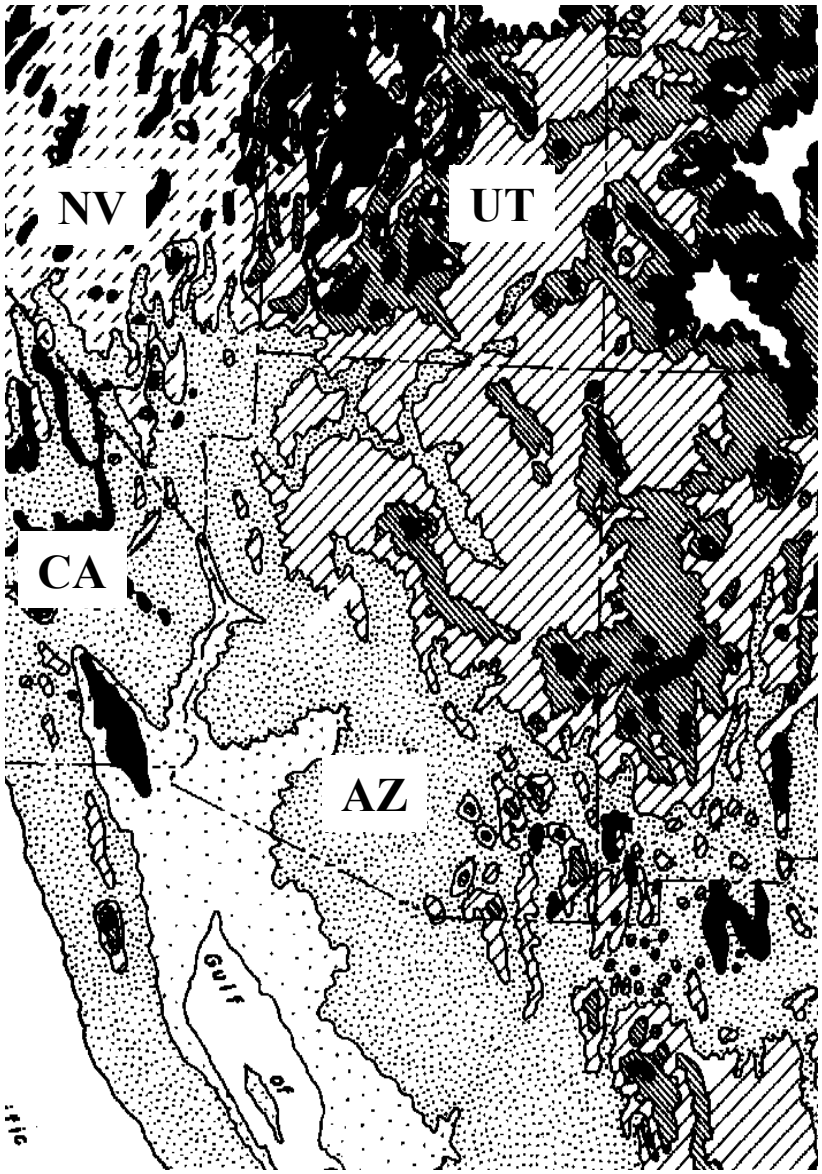


Figure 12. Modified figure from Betancourt et al. (1990) illustrating the extent of the warm desert refugia (light stippling) to the southern tip of Nevada during the Pleistocene.

^{14}C yr BP (radio carbon date years before the present) (Hockett, 2000). Lizards such as Collared Lizards (*Crotaphytus collaris*), Leopard Lizards (*Gambelia wislezini*), Whip-tailed Lizards (*Cnemidorphorus* sp.), and Desert Horned Lizards (*Phrynosoma platyrhinos*)that occur in the present day community in both warm and cold desert habitat are found throughout the strata all the way to 32,000 ^{14}C yr BP (Hockett, 2000). Chuckwalla remains are not found in the Pintwater Cave record until about 10,100 ^{14}C yr BP.

The phylogeny also agrees with what is observed in color pattern variation among populations. On closer examination, color patterns in the northern clade are merely a variation on the same basic striped dorsal color pattern. Some populations are darker than others with faint hints of stripes, while others are more obvious (Figure 13). Our observations on color pattern at this small scale agree with what Hollingsworth (1998) found over a broader geographic range.

Since there is good fossil evidence that chuckwallas are relatively recent arrivals to the mountain ranges outside of the Colorado River trough in Nevada, we attribute the lack of resolution in the phylogenetic analysis to this recent arrival. The great amount of unique haplotypes recovered reflect lack of present gene flow as indicated by their specific habitat preferences and life history characteristics that may be conducive population differentiation (Johnson, 1965; Berry, 1974; Abts, 1987).

There is evidence that chuckwallas have already diverged with respect to life history characteristics (Tracy, 1999). In a common garden experiment, Tracy (1999) raised juvenile chuckwallas in the lab and found that animals from different populations had different growth rates. The differences could be roughly explained by elevation and variation in rain fall. Genetic divergences at the gene fragments examined in this study have not diverged at the same detectable rate.

This lack of resolution in the phylogenetic analysis could also be because populations are currently experiencing gene flow. There is some evidence that continuing gene flow may be an important force in at least some instances. For example, for the Goodsprings population, we not only detected intrapopulation genetic differences, but also found that two of these individuals grouped more closely to chuckwallas from the Specter Range. This result indicates that the two divergent Goodsprings lizards contained haplotypes more genetically similar to chuckwallas from other populations than to individuals in their own population. In addition, one cytochrome *b* haplotype was found in 37 individuals distributed among a centrally located cluster of eleven adjacent mountain ranges. While there were fewer mountain ranges with individuals sharing control region haplotypes, they were found within this cluster of mountain ranges as well.

It is also possible that the lack of resolution was a result of the possible errors for phylogeny estimating at the population level (Templeton, et al. 1992). Taking into account the historical ecology of this region, chuckwalla life history characteristics that suggest low present day gene flow, and the phylogenetic pattern we recovered we hypothesize that a population level analysis should reveal little present day gene flow. What follows are the results of the TCS population level analysis.

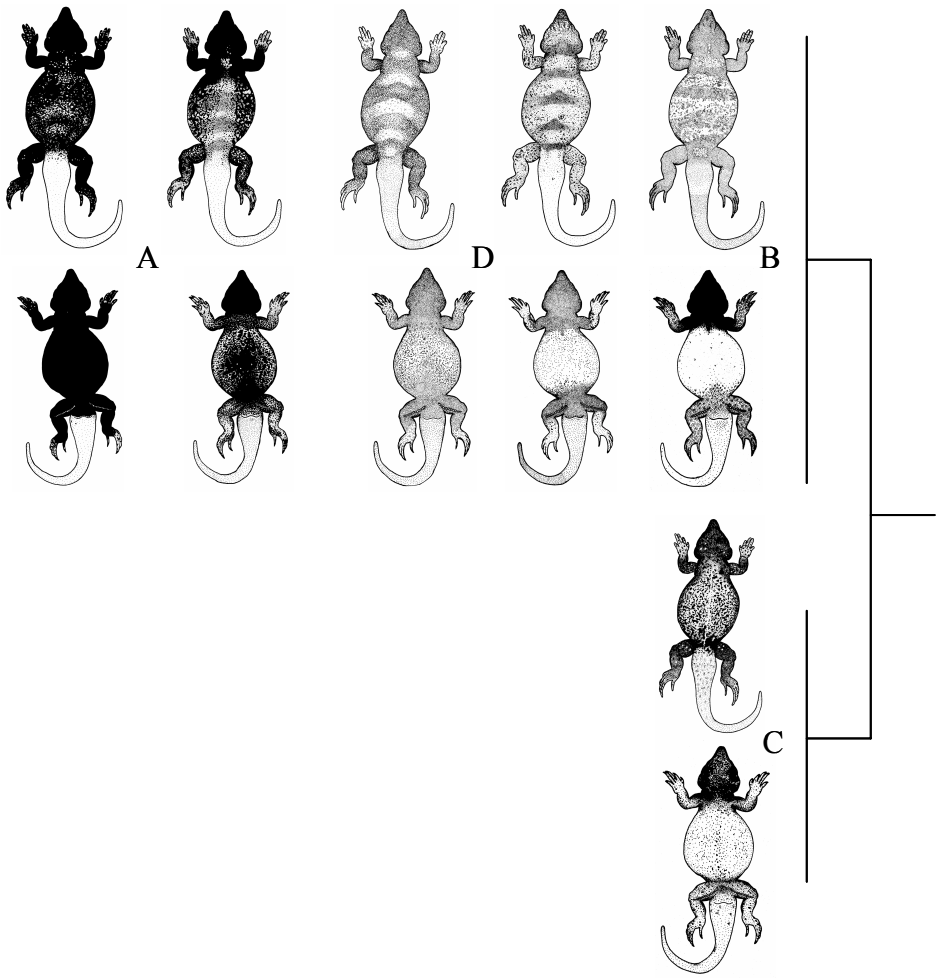


Figure 13. Color pattern congruence with northern Mojave "striped" clade and southern "Newberry" clade. A) Black; B) Eldorado; C) Newberry Mountains; and C) Virgin River. Dorsal color pattern is represented in the top row within each clade and ventral color pattern is represented in the bottom row.

Recent colonization but no current gene flow

As an aid in interpreting the results, Figure 14 presents a rough overlay of the respective mtDNA fragment cladograms over geography. The analysis revealed significant nonrandom association of clades and sampling locations, indicating phylogeographic structure in the data at higher clade levels. Geographic distributions of clades indicate three well-supported population fragmentation events: restricted gene flow with isolation by distance, restricted gene flow/dispersal but with some long distance dispersal, and contiguous range expansion. Restricted gene flow is found in different extents among haplotypes nested throughout the network. The data sets from the analysis of cytochrome *b*, control region, and both combined fragments all show a pattern of some level of restricted gene flow at haplotype and lower nesting levels followed by range expansion at higher levels (see Appendix G). The interpretation of these data imply chuckwalla populations are relatively recent arrivals (geologically speaking) and currently experience relatively little or no gene flow among populations. This suggests that mountain ranges sampled in this study hold unique populations that may be on different evolutionary trajectories.

The basic pattern for restricted gene flow is for tip clades and haplotypes to be more geographically restricted than interiors, for tips to be scattered throughout the range of the interior clades, and for these patterns to keep occurring at higher and higher clade levels unless geographical homogeneity is achieved (Templeton et al, 1995). All of these patterns are evident in Figures 15–23 and restricted gene flow via isolation by distance was inferred for many of the clades (Tables 4–12). These statistical inferences are concordant with what is known about the biology of chuckwallas. Recruitment has largely been recorded from within populations (Abts, 1987). An isolation by distance model of gene flow is to be expected from this species and such was detected.

The basic pattern for range expansion is for older (interior) haplotypes to be left in the ancestral area while younger (tip) haplotypes that originated in the expanding population can be geographically widespread and/or distant from their ancestral haplotypes (Templeton et al, 1995). Several separate instances of range expansion were inferred for these chuckwalla samples. Each haplotype analysis revealed a common, widespread haplotype located among the mountains that drain into the Muddy and Virgin rivers (XV for cytochrome *b*; XVIII for control region; XLIII for combined). Younger haplotypes radiate geographically from these interior haplotypes into two other clades. One consisting of haplotypes from the Eldorado and McCullough Mountains (Clade 2-3 for cytochrome *b*; Clade 4-3 for control region; Clade 4-3 for combined) and the other consisting of haplotypes west of the river drainages (Clade 2-1 for cytochrome *b*; Clade 4-4 for control region; Clade 5-1 for combined). The cytochrome *b* data set detected range expansion between the two largest clades. These types of patterns are consistent with the packrat midden data that implies chuckwallas have only been in the northern Mojave since the last 10,000 years.

The patterns observed in this study seem to confirm the hypothesis that chuckwallas are recent post-Pleistocene immigrants to the habitat of the northern Mojave Desert but are currently experiencing little or no gene flow. All three data sets also show a pattern of a geographically widespread interior or ancestral haplotype occurring among the mountain ranges that drain into the Muddy and Virgin rivers. Younger haplotypes

Figure 14. The haplotype network for the A) cytochrome b region haplotypes; B) control region haplotypes; and C) combined cytochrome *b* and control region haplotypes as estimated for *Sauromalus obesus* overlaid on their geographic location. Each line in the network represents a single, unambiguous mutational change. Black dots indicates an interior node in the network that was not present in the sample; that is, these are inferred intermediate haplotypes between two nearest neighbor haplotypes in the network that differed by two or more mutations. Size of haplotype circles and squares is roughly proportional to haplotype frequency. Squares represent haplotypes with the greatest outgroup probability. Roman numerals represent unique haplotypes and Arabic numerals are the localities where the unique haplotypes are found. Locality numbers are listed in Figure 7.

radiate from this haplotype into the surrounding sampled areas. This suggests that chuckwallas could have moved north from the Colorado River trough (the southern refugia 10,000 ybp) along the Virgin and Muddy River courses. Both of these rivers flow from the north into the Colorado River. Clades and haplotypes at lower levels of nesting show patterns of restricted gene flow. As clades are pooled progressively higher in the analysis, it is revealed that these populations never the less are closely related because they are linked by contiguous range expansion.

Two instances in this analysis illustrate the importance of geographical and sampling design. First, the range expansions found for most of the clades were specifically inferred to be a contiguous range expansion. In contrast, it was not possible to discriminate between contiguous range expansion and long-distance colonization for the expansion occurring from populations 1 (Alkali Flat) and 2 (Bonnie Claire Flat). These differences reflect the fact that both of these populations were sampled from small isolated rocky outcrops located within valleys more than several kilometers from the adjacent mountain ranges. While the haplotypes from these populations are definitely unique, it is necessary to sample the adjacent mountain ranges between them to resolve their relationship. When sampling is incomplete in an area, it becomes impossible to discriminate between short and long-distance movements (Templeton et al, 1995). Second, past fragmentation was inferred for control region haplotypes nested in Clades 2-3 and 2-7. Both of these clades contain haplotypes from the anomalous Goodsprings (18) population. Individuals from this population appear in both major clades in all analyses. The relationship among the Goodsprings haplotypes could be less ambiguous if more sampling occurred west of this population in California. Goodsprings is right on the western border of Nevada. A more remote possibility could be human facilitated movement of chuckwallas among localities across natural barriers to dispersal. Native Americans infrequently captured *Sauromalus obesus* for subsistence. Gifford (1936) reported that the northeastern and western Yavapai collected chuckwallas for food. Jaeger (1950) observed southern Paiutes in southern Nevada preparing chuckwallas to eat, and Steward (1941) documented the chuckwalla hunting techniques of Death Valley Native Americans. Since the capture of chuckwallas was infrequent and incidental, it is unlikely that Native American hunts had a significant effect on chuckwalla population numbers. However, Steward (1941) reported that chuckwallas were sometimes traded to neighboring groups that came from areas where chuckwallas were scarce or did not occur. Whether the animals were traded dead or alive was not mentioned. The delivering of live chuckwallas across natural barriers of dispersal could have had an effect on the range and genetic structure of present chuckwalla populations. Hence, before strong inferences can be made about the forces that explain the geographical distribution of genetic variation, adequate geographical sampling must be taken into account, and less obviously, recent human influence could be a factor especially in an animal confirmed to be used as a resource. While Native Americans' were undeniably less obtrusive compared to people derived from European cultures, there is a tendency to discount any influences they may have had on presumed "undisturbed" natural populations.

The statistical inference structure presented here is designed to geographically and cladistically identify the effects of restricted gene flow and historical events on geographical associations of haplotypes (Templeton et al, 1995). This analysis inferred two major events for chuckwalla populations surveyed in this study: a recent post-Pleistocene contiguous range expansion from southern Sonoran refugia north into the

Mojave by way of the Muddy and Virgin river drainages. This was followed by local differentiation because of reduced present day gene flow.

RECOMENDATIONS

Population studies are perhaps most pertinent to state wildlife management agencies since these institutions have jurisdiction over plant and animal populations that happen to fall within the state's political boundaries rather than the species as a whole. Chuckwallas range well outside of the political boundaries of Nevada so it is important for Nevada managers to know how chuckwalla populations are structured within Nevada. This is even more pertinent since the critical evolutionary and ecological functional unit is not necessarily the species, but the population (Meffe and Carroll, 1994). The local population is where responses to environmental challenges occur, where adaptations arise and where genetic diversity is maintained and reshuffled each generation. A wide ranging taxon such as chuckwallas may consist of many genetically isolated or semi-isolated populations that play different functional roles in Nevada than they may for populations in Arizona for example. Geographic variation is an important element in both the study of evolutionary processes and the practice of conservation biology because it reflects local adaptations, and is a first step towards the process of speciation, the development of new species. It is itself a form of biological variation worthy of protection and perpetuation (Meffe and Carroll, 1994).

It has already been shown that some chuckwalla populations have evolved different growth rates and male color patterns depending on the availability of forage particular to the population (Tracy, 1999; Kwiatkowski and Sullivan, 2002*a,b*). Genetically based plasticity in life history characters is a pervasive feature of most organisms. If a population of chuckwallas is lost or critically reduced within a mountain range, for example, it does no good to the rest of the local species that depend on the chuckwalla if chuckwalla populations exist elsewhere. Unless recolonization from elsewhere can occur, this population extinction is as important functionally to that local system as if the entire species were destroyed. Population persistence within each local system is more important than simple overall species persistence.

The genetic analysis of Nevada chuckwalla populations found that chuckwalla populations in Nevada do indeed contain unique genetic and phenotypic traits and any threat to a population would represent a decline in the biodiversity even if populations persist elsewhere. We found chuckwallas to be distinct genetically among the maintain ranges we surveyed within Nevada. In addition, the removal study implied that chuckwalla populations affected in the manner of this study may be slow to return to pre-removal observations. All but one of the populations removed experienced a decrease in the number of chuckwallas observed in subsequent seasons. This is consistent with what is known about chuckwalla biology. Chuckwallas are relatively long lived lizards, females have small clutches of proportionally large eggs, experience little or no detectable migration, and are habitat specialists (Johnson, 1965; Berry, 1974; Abts, 1987). In addition, high adult survivorship, low juvenile survivorship, low recruitment, and infrequent breeding don't appear to be traits that can withstand the large scale removal of breeding adults from the populations. All of these factors contribute to an organism that one would suspect is susceptible to slow recovery rates after experiencing a population crash. Chuckwallas may not be imperiled by commercial collection as a species (and maybe not even within the political boundaries of Nevada as a whole) but it is indeed possible for local populations to be adversely affected. While chuckwallas as a species may not be under threat in Nevada, there is good evidence for the potential loss of

populations or at least the erosion of genetic diversity. Chuckwallas may be adapted to conditions particular to the mountain range they occupy and there is little evidence of migration among populations. The removal of individuals may have an adverse effect on the specialized adaptations and localized gene pools.

Knowledge of levels of genetic variation within populations may be important in conservation efforts if levels of genetic diversity influence current or future persistence of populations (Elam, 1998). Reduced levels of variation may decrease the potential for persistence in the face of long-term biotic or abiotic environmental change or short-term impacts such as collectors or pathogens (Meffe and Carroll, 1994). Chuckwallas are adapted to the subtropical-like conditions of the desert spring and are heavily dependent on the spring crop of annuals (Berry, 1974). In times of drought, populations have been documented to decrease by large amounts in short intervals in time (Abts, 1987). Mangel and Tier (1994) refer to such changes as "catastrophes" and include physical factors such as "hurricanes, freezes, and droughts, biological factors such as epidemics or invasion by a new competitor or predator, or perturbations of the environment caused by humans." They stress the importance of including catastrophes in population viability models because it forces us to think differently about the evaluation of conservation measures (Mangel and Tier, 1994). At a local level, "catastrophes are more likely to make local extinction far more common than short-term studies of environmental variability would lead us to believe" (Mangel and Tier, 1994). An animal that is adapted to natural catastrophes may not be able to handle a human caused catastrophe (over collecting).

Given that estimating chuckwalla numbers in the field is difficult we suggest that it is perilous to continue to resume collecting pressure on their populations. It is known that chuckwallas exhibit life history characteristics that make them particularly vulnerable to population disturbances and local populations may be slow to recover given the observations of the experimentally removed populations in this study. Given the amount of genetic distinctiveness we recovered, estimating the numbers that are there may be irrelevant. We believe it is more critical to protect local populations and the genetic diversity contain within. Chuckwalla abundance appears to be a function of the quality of the rocks that contain suitable crevices for retreat and rock piles that provide basking sites. It appears that the populations in the Newberry Mountains may harbor the greatest densities of chuckwallas per unit area compared to populations in other mountain ranges in Nevada. This is also where most of the animals in the state have been collected historically. This is also a population where we observed some of the greatest genetic diversity in Nevada. Continued collection in this area could contribute to the erosion of genetic diversity unique to this population.

The genetic analysis suggests that chuckwalla populations among mountain ranges in Nevada may have the potential to be on separate evolutionary trajectories. Color pattern and mtDNA haplotypes may not reflect the extent to which ecological and life history characteristics have already evolved. In a harsh desert environment such as the northern Mojave, life history and physiological traits may be under greater selective pressure than color pattern. Case (1976) found differences in body sizes across elevation and the amount of rainfall. Tracy (1999) found in common garden experiments that juvenile chuckwallas from different populations had different growth rates. Kwiatkowski and Sullivan (2002*a,b*) observed variation among mountain ranges in the life history traits they measured. In the classic studies of chuckwalla natural history, Johnson (1965), Nagy (1973), Barry (1974), and Abts (1987) all observed population demographics that differed

widely. Deducing solely from other life history studies on chuckwallas in the Mojave it appears that their biology is such that it might not be well equipped to withstand commercial collection pressures at the local level.

Chuckwallas exhibit many population characteristics that maintain high levels of genetic differentiation among populations. Since males cultivate many females to mate with, the effective population sizes are continually small in size (Kwiatkowski and Sullivan, 2002*a,b*). Populations fluctuate in size over time (Barry, 1972 and Johnson, 1966). The level of gene flow among populations is very low (hence populations are very isolated) and there is evidence that selection may cause the development of geographic races (Tracy, 1998; Kwiatkowski and Sullivan, 2002*a,b*).

If populations of chuckwallas in Nevada have high levels of among-population genetic variation as our data suggest and have been shown to vary among populations in evolutionary significant characters (e.g. Tracy, 1998 and Kwiatkoski and Sullivan, 2002*a,b*), then distinct populations among mountain ranges in Nevada need to be preserved to ensure adequate representation of allelic and genotypic diversity within the taxon. We suggest maintenance of the distinct populations among mountain ranges in Nevada is necessary to preserve species level genetic variation within Nevada.

We also suggest that even if collectors follow all the regulations imposed on them by the state of Nevada, allowing for the commercial collection of chuckwallas creates the opportunity for collectors from other states to launder their illegally collected chuckwallas. In a random search at a pet trade show in Texas, we found a chuckwalla for sale that was advertised as wild caught in Nevada. Based on our experience with the color pattern characteristics for chuckwallas found in Nevada, we concluded that it was highly improbably that this chuckwalla came from the state of Nevada. This particular animal did not share any of the color pattern characteristics from any of the populations we surveyed in Nevada. The dealer himself even doubted that the animal was from Nevada. Comparing this chuckwallas' mtDNA haplotype to chuckwallas known to be collected from Nevada would be interesting.

Useful information collected from the rest of this study should concentrate on characterizing the limiting factors that effect sizes of breeding pools, the maintenance of genetic diversity, and the effect of environmental states on growth and development. An increased understanding of the biology of Nevada chuckwallas will allow better decisions about what kind of hits populations can take from commercial collecting.

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Appendix A

Distance from chuckwalla basking site to crevice before capture and distances (di) to nearest shrub for each quadrant using the chuckwalla basking location as the center point (distances in meters).

Dist. to crevice (m)	d1	d2	d3	d4	Elevation (m)	Slope (˚)	Aspect (˚)
2.00	4.0	3.5	4.0	0.8	540	30	220
1.20	4.0	6.0	2.2	2.3	540	30	220
1.00	0.6	0.4	0.4	3.4	540	30	220
0.12	1.0	0.5	5.0	0.3	540	30	220
0.30	0.3	0.4	2.0	6.0	540	30	220
0.30	0.3	0.4	2.0	6.0	540	30	220
0.00	1.0	4.0	0.5	1.5	1020	22	178
0.04	0.6	4.0	1.4	1.0	1020	22	178
1.00	2.0	2.3	1.1	0.3	1020	22	178
0.50	1.0	9.0	12.0	3.0	1020	22	178
2.50	1.0	2.2	2.0	5.0	1020	22	178
0.00	9.0	1.2	1.3	3.3	1200	22	136
0.00	9.0	1.2	1.3	3.3	1200	22	136
3.00	10.0	7.0	11.0	14.0	840	25	182
1.30	0.6	0.4	0.8	1.3	840	25	182
1.20	8.0	6.0	1.0	1.3	840	25	182
0.30	4.0	6.0	7.0	8.0	840	25	182
0.30	2.0	1.0	1.3	3.3	840	25	182
1.00	2.2	8.0	9.0	10.0	960	25	174
0.20	3.2	5.0	4.0	2.0	960	25	174
0.13	3.0	4.0	2.2	2.3	960	25	174
0.10	0.1	22.0	4.0	8.0	960	25	174
0.40	n/a	n/a	n/a	n/a	960	25	174
0.15	n/a	n/a	n/a	n/a	960	25	174
0.12	n/a	n/a	n/a	n/a	960	25	174
0.00	4.0	12.0	3.0	2.1	1110	22	180
0.00	1.8	1.9	1.5	2.4	540	36	186
1.40	3.5	2.5	2.5	1.2	990	18	220
0.50	5.0	3.5	1.8	2.3	990	18	220
0.90	1.2	1.4	1.1	1.3	990	18	220
0.40	1.8	1.2	4.9	1.5	990	18	220
2.50	2.6	1.7	2.9	3.7	990	18	220
0.10	0.9	2.0	1.2	1.4	990	18	220

Dist. to crevice (m)	d1	d2	d3	d4	Elevation (m)	Slope (°)	Aspect (°)
2.60	0.9	2.2	2.0	0.8	990	18	220
0.90	2.7	3.0	1.4	2.4	990	18	220
0.30	2.3	2.0	2.8	1.6	990	18	220
0.30	3.1	2.4	2.6	4.4	1230	33	130
0.40	3.2	4.2	1.8	2.3	1230	33	130
0.80	0.8	2.8	3.1	1.6	1230	33	130
1.10	1.7	3.1	2.2	1.7	1230	33	130
1.40	1.1	1.7	1.7	1.5	810	29	200
1.10	2.1	2.8	2.2	2.1	810	29	200
0.00	0.8	1.2	1.4	1.7	810	29	200
1.60	1.2	1.3	0.3	1.5	810	29	200
0.10	0.7	1.9	1.1	1.6	810	29	200
1.00	1.3	1.7	1.4	1.5	810	29	200
1.50	0.6	1.6	1.2	1.5	1110	22	180
0.50	1.1	1.4	2.1	0.3	1110	22	180
0.40	0.6	1.1	1.4	1.3	1110	22	180
1.40	0.8	1.9	1.0	0.4	900	18	138
1.00	2.2	2.1	1.0	1.6	900	18	138
1.10	n/a	n/a	n/a	n/a	900	18	138
0.60	n/a	n/a	n/a	n/a	900	18	138
0.60	1.2	1.3	1.8	1.2	1230	33	140
0.90	2.0	1.1	0.9	1.6	1110	22	180
0.40	1.8	0.7	0.6	1.3	1110	22	180
0.30	0.7	0.6	1.2	1.4	1050	19	168
0.80	0.8	0.5	1.2	1.4	1050	19	168
0.40	1.6	1.2	0.9	2.5	1050	19	168
2.60	0.4	2.4	2.4	0.7	1350	32	132
2.10	2.7	4.0	2.6	2.0	1350	32	132
12.30	2.0	3.8	2.2	2.3	1350	32	132
0.30	1.2	1.6	2.6	2.1	1350	32	132
0.50	1.1	1.5	2.1	1.5	1350	32	132
1.20	1.2	0.6	2.8	1.5	1000	24	264
1.10	2.2	2.3	4.0	2.5	1000	24	264
1.30	2.2	2.9	1.3	1.5	1000	24	264
1.20	0.5	2.9	1.8	1.0	1000	24	264
1.50	3.7	3.5	1.8	1.6	1000	24	264
8.30	1.6	2.2	2.3	2.8	1230	33	140
0.70	0.6	1.7	1.1	0.9	1230	33	140
0.00	0.8	0.2	1.1	0.8	1230	33	140
1.70	3.1	5.8	2.3	2.6	540	36	186

Dist. to crevice (m)	d1	d2	d3	d4	Elevation (m)	Slope (°)	Aspect (°)
0.10	2.0	3.3	1.9	2.3	540	36	186
1.20	0.3	2.6	3.4	3.8	540	36	186
0.90	2.3	3.1	1.2	1.1	540	36	186
0.30	1.3	1.8	6.7	3.7	650	22	154
0.80	2.2	2.9	3.6	2.7	1170	31	160
1.50	1.9	1.6	1.2	1.0	1170	31	160
0.10	1.2	2.1	3.2	2.1	1170	31	160
1.00	1.3	3.6	3.0	1.3	1170	31	160
0.10	2.5	4.1	5.7	8.2	1170	31	160
Mean = 1.04					971	26	181
Range = 0–12.3					540–1350	18–36	130–264
STDV = 1.66					225	5.6	35.8

Appendix B

Field number; sex (juv=juvenile), weight in grams, and snout-vent length in millimeters of chuckwallas removed at each site.

SITE	FIELD NO.	SEX	WT(g)	SVL(mm)	TL(mm)
V-1	PCU 259	M	270	197	400
	PCU 260	F	120	152	287
	PCU 261	juv	15	NA	NA
	PCU 262	juv	15	NA	NA
	PCU 277	F	81	136	260
	PCU 278	juv	17	76	158
	PCU 285	F	95	151	NA
	PCU 286	M	155	173	360
	PCU 292	F	155	143	287
	V-1	M	250	185	365
	V-10	M	240	193	390
	V-11	juv	45	105	210
	V-12	M	215	182	325
	V-13	M	300	200	357
	V-14	F	180	160	310
	V-15	M	225	180	349
	V-16	M	260	182	385
	V-17	F	155	155	270
	V-18	M	250	183	343
	V-19	M	230	175	352
	V-2	M	255	191	397
	V-20	M	290	295	405
	V-21	M	45	112	223
	V-22	M	245	275	355
	V-23	M	330	299	424
	V-24	M	60	124	245
	V-25	F	175	160	305
	V-26	M	245	180	362
	V-27	M	250	282	370
	V-28	M	100	132	208
	V-29	M	235	174	360
	V-3	M	220	187	378
	V-30	M	205	166	342
	V-31	M	305	202	405
	V-32	M	255	188	385
	V-33	F	170	160	281
	V-34	M	340	198	367
	V-35	F	235	177	302
	V-36	M	75	123	252
	V-37	F	50	116	230
	V-38	F	50	117	226
	V-39	M	120	117	304
	V-4	M	300	201	336

SITE	FIELD NO.	SEX	WT(g)	SVL(mm)	TL(mm)
	V-5	M	135	152	282
	V-6	M	135	178	282
	V-7	M	280	192	384
	V-8	M	305	205	327
	V-9	F	150	147	300
	VM-1	F	223	178	320
	VM-2	M	260	187	344
	VM-3	M	347	213	435
V-2	PCU 608	M	260	210	410
	PCU 612	M	50	115	220
	PCU 708	M	295	201	323
	PCU 709	M	320	199	322
V-3	PCU 604	F	227	185	333
	PCU 605	F	45	115	218
	PCU 606	F	125	150	310
	PCU 607	F	42	105	190
	PCU 657	F	95	141	288
	PCU 658	M	290	197	405
	PCU 659	M	300	197	336
	PCU 660	M	200	176	342
	PCU 661	F	85	130	246
	PCU 662	M	290	200	357
	PCU 663	M	285	198	389
	PCU 664	F	47	113	215
	PCU 665	F	40	109	205
	PCU 666	F	31	99	187
	PCU 667	F	57	116	228
	PCU 668	F	165	166	315
	PCU 669	M	155	168	330
	PCU 670	M	320	206	421
	PCU 671	F	66	126	247
	PCU 672	M	250	183	363
NB-1	NB-52	M	130	146	280
	NB-53	M	145	152	319
	NB-54	M	220	167	294
	NB-55	M	300	206	420
	NB-56	F	150	145	245
	NB-57	juv	35	111	180
	NB-58	F	150	160	310
	NB-59	juv	55	118	225
	NB-60	M	190	177	367
	NB-61	M	55	120	264
	NB-62	M	65	126	237
	NB-63	M	80	133	264
	NB-64	M	75	131	268
	NB-65	M	130	152	265
	NB-66	M	45	110	185
	PCU 322	M	176	159	254
	PCU 556	juv	60	121	234

SITE	FIELD NO.	SEX	WT(g)	SVL(mm)	TL(mm)
	PCU 557	juv	40	111	169
	PCU 558	F	115	155	240
	PCU 559	M	190	190	329
	PCU 560	juv	22	74	138
	PCU 561	juv	70	124	213
	PCU 562	M	175	190	381
	PCU 563	F	63	120	241
	PCU 566	F	145	178	271
	PCU 567	F	147	165	320
	PCU 568	F	120	160	308
	PCU 569	F	147	176	332
	PCU 617	M	185	174	312
	PCU 619	F	140	154	308
	PCU 620	M	145	161	320
	PCU 621	M	200	179	354
NB-2	PCU 693	M	232	179	338
	PCU 694	M	188	167	344
	PCU	NA	Not Available(NA)	NA	NA
	PCU	F	NA	NA	NA
	PCU 570	M	130	160	286
	PCU 571	M	65	123	177
	PCU 572	M	163	175	365
	PCU 573	F	118	154	267
	PCU 574	M	212	192	360
	PCU 579	NA	45	NA	NA
	PCU 580	NA	150	NA	NA
	PCU 581	NA	35	NA	NA
	PCU 594	NA	10	NA	NA
	PCU 695	M	309	200	335
	PCU 696	F	89	134	270
	PCU 697	F	134	149	291
	PCU 698	juv	25	95	178
	PCU 699	F	105	145	280
	PCU 700	F	126	152	275
	PCU 701	F	197	175	332
	PCU 702	F	115	140	276
NB-3	NB-1	F	65	115	225
	NB-10	M	175	161	315
	NB-11	F	170	175	318
	NB-12	M	200	171	314
	NB-13	M	245	180	361
	NB-14	M	140	148	249
	NB-15	M	205	176	348
	NB-16	F	85	125	215
	NB-17	juv	90	130	253
	NB-18	juv	40	99	191
	NB-19	M	245	179	332
	NB-2	M	235	180	358
	NB-20	M	100	130	230

SITE	FIELD NO.	SEX	WT(g)	SVL(mm)	TL(mm)
	NB-3	F	155	151	243
	NB-4	M	150	155	288
	NB-5	juv	30	94	183
	NB-50	M	65	119	223
	NB-51	juv	50	112	234
	NB-6	juv	40	101	199
	NB-7	M	150	159	293
	NB-8	M	180	172	315
	NB-9	M	165	163	285
	PCU 263	F	90	144	273
	PCU 264	F	80	146	296
	PCU 265	F	55	118	221
	PCU 266	F	55	132	271
	PCU 267	M	95	142	270
	PCU 268	M	40	109	189
	PCU 269	juv	10	68	130
	PCU 270	juv	10	69	134
	PCU 272	M	130	170	339
	PCU 273	M	115	146	286
	PCU 274	F	80	141	264
	PCU 275	F	65	135	247
	PCU 276	F	55	119	171
	PCU 280	F	80	141	283
	PCU 281	M	110	159	289
	PCU 284	M	130	166	331
BC-1	BC-1	F	315	193	305
	BC-2	M	175	156	302
	BC-20	M	175	166	337
	BC-21	M	400	212	335
	BC-22	M	160	162	321
	BC-4	F	300	190	303
	PCU 188	M	255	185	393
	PCU 279	M	325	214	435
BC-2	PCU 582	M	NA	201	303
	PCU 583	M	180	171	261
	PCU 584	M	150	167	325
	PCU 585	M	150	180	342
	PCU 586	M	195	180	322
	PCU 587	F	150	170	311
	PCU 588	F	95	152	277
	PCU 589	F	85	146	272
	PCU 590	juv	12	68	125
	PCU 627	M	265	193	361
	PCU 628	F	130	156	270
	PCU 629	M	145	156	252
	PCU 630	F	145	161	305
	PCU 631	M	250	182	322
	PCU 632	F	120	157	295
	PCU 633	M	285	191	384

SITE	FIELD NO.	SEX	WT(g)	SVL(mm)	TL(mm)
	PCU 634	juv	11	71	132
	PCU 635	M	230	193	356
	PCU 636	F	115	149	276
	PCU 637	F	120	162	309
	PCU 638	F	130	155	291
	PCU 639	juv	12	66	114
	PCU 640	M	135	156	295
	PCU 641	M	260	191	377
BC-3	PCU 598	NA	225	NA	NA
	PCU 599	NA	115	NA	NA
	PCU 600	M	240	200	355
	PCU 601	M	235	205	320
	PCU 602	F	105	155	300
	PCU 603	M	200	187	368
	PCU 711	M	320	200	380
	PCU 712	M	210	175	350
	PCU 713	M	381	198	300
	PCU 714	M	350	203	406
	PCU 715	M	330	201	395
	PCU 716	M	255	182	364
	PCU 717	M	270	194	346
	PCU 718	F	220	181	349
	PCU 719	F	215	175	304
	PCU 720	M	350	192	270
	PCU 721	M	240	182	364
	PCU 722	M	280	196	383
	PCU 723	M	393	196	290
	PCU 724	F	205	170	319
	PCU 725	F	216	115	52
	PCU 726	M	415	202	350

Appendix C

Specimens for which mtDNA sequence data were collected for this study.

Sauromalus obesus: USA: ARIZONA: MOHAVE COUNTY: Gila Mts., N 32˚ 26'00.00" W 114˚ 09'30.00", 350 m, PCU 731. NEVADA:CLARK CO.: Arrow Canyon Range, N 36˚ 32'10.62" W 114˚ 54'35.28", 810 m, PCU 401–403, 405–406; Eldorado Mts., N 35˚ 42'32.22" W 114˚ 47'35.34", 540 m, PCU 330–335; McCullough Mts., N 35˚ 44'41.46" W 115˚ 08'16.62", 1020 m, PCU 338–342; Muddy Mts., Buffington Pockets, N 36˚ 23'04.80" W 114˚ 41'28.68", 900 m, PCU 359–365; Newberry Mts., N 35˚ 10'27.60" W 114˚ 41'54.18", 715 m, PCU 271; N 35˚ 07'18.00" W 114˚ 41'21.18", 500 m, PCU 264, 267, 280, 281, 288, 289, 295, 297; North Muddy Mts., Hwy 169 W of west entrance to Valley of Fire State Park, N 36˚ 24.53'.82" W 114˚ 36'26.64", 945 m, PCU 213; River Mts., N 36˚ 05'52.74" W 114˚ 54'10.50", 540 m, PCU 376–377, 482–484; Sheep Range, road cut 1.0 mi E of I15/NV 604 junction on 604, N 36˚ 19'19.02" W 114˚ 56'46.98", 650 m, PCU 486; Spotted Range, N 36˚ 34'24.72" W 115˚ 50'57.30", 1050 m, PCU 410–411, 460–462; Spring Mts., E of Sandy on Sandy Valley Road, between Sandy and Goodsprings, N 35˚ 48'22.62" W 115˚ 31'00.12", 1200 m, PCU 343, 346–350; Indian Ridge, N 36˚ 29'35.58" W 115˚37'21.54", 1230 m, PCU 396–399; Virgin Mts., N 36˚ 31'53.04" W 114˚ 09'56.46", 845 m, PCU 260, 285–287, 290; ESMERALDA CO.: Bonnie Claire Flat, N 37˚ 08'50.70" W 117˚ 10'55.50", 1260 m, PCU 188; N 37˚ 09'07.32" W 117˚ 10'51.54", 1232 m, PCU 291, 298; LINCOLN CO.: Delamar Mts., N 37˚ 02'18.36" W 114˚ 51'01.86", 900 m, PCU 443; East Mormon Mts., N 36˚ 52'29.88" W 114˚ 21'27.24", 1028 m, PCU 433–434; East Mormon Mts., N 36˚ 52'24.48" W 114˚ 22'09.66", 1110 m, PCU 455–456; Hiko Range, Hell's Half Acre, SE town of Ash Springs, N 37˚ 28'31.74" W 115˚ 10'01.68", 1400 m, PCU 181; Meadow Valley Mts., N 37˚ 00'12.90" W 114˚ 52'49.02", 919 m, PCU 237; Meadow Valley Mts., N 37˚ 01'08.70" W 114˚ 51'12.06", 900 m, PCU 438–441; Mt. Irish Range, Fossil Peak, N 37˚ 41'33.30" W 115˚ 11'18.36", 1230 m, PCU 479–481; Rainbow Canyon, 19.7 mi S of 93/317 junction on 317, N 37˚ 22'01.86" W 114˚ 32'54.24", 1170 m, PCU 487–491; Tikaboo Valley, around 1.0 mi N of Nellis Air Force Range boundary fence, N 37˚ 17'49.56" W 115˚ 26'42.24", 1260 m, PCU 227; NYE CO.: Alkali Flat/Stonewall Mt., N 37˚ 25'16.86" W 117˚ 07'46.98", 1350 m, PCU 464–468; Black Marble Mt., N 36˚ 45'21.06" W 116˚ 37'27.66", 1000 m, PCU 474, 476–478; SW slope of Black Marble from Steve's Pass, N 36˚ 45'25.86" W 116˚ 37'31.32", 1110 m, PCU 184; Last Chance Range, N 36˚ 12'51.78" W 116˚ 07'55.92", 840 m, PCU 352–357; Specter Range, N 36˚ 37'13.50" W 116˚ 17'22.98", 990 m, PCU 389, 391–395. UTAH: WASHINGTON CO.: SW slope of Beaver Dam Mts., N 37˚ 05'22.20" W 113˚ 56'11.46", 1202 m, PCU 192

Appendix D

Field number, locality, and haplotype labels.

PCU #	Locality	Cyt *b* haplotype	Control region haplotype	both
464	Alkali Flat	A	A	1
465	Alkali Flat	A	A	1
466	Alkali Flat	A	A	1
467	Alkali Flat	A	A	1
468	Alkali Flat	A	A	1
188	Bonnie Claire Flat	A	F	3
291	Bonnie Claire Flat	A	F	3
298	Bonnie Claire Flat	A	F	3
181	Hiko Range	AA	R	4
184	Black Marble Mtn.	B	E	5
474	Black Marble Mtn.	B	E	5
476	Black Marble Mtn.	B	E	5
477	Black Marble Mtn.	B	E	5
478	Black Marble Mtn.	B	E	5
192	Beaver Dam Mts.	C	AN	6
441	Meadow Valley Mts.	D	AA	7
360	Muddy Mts.	D	AB	8
361	Muddy Mts.	D	AC	9
362	Muddy Mts.	D	AC	9
365	Muddy Mts.	D	AC	9
213	North Muddy Mts.	D	AD	10
487	Rainbow Canyon	D	AF	11
488	Rainbow Canyon	D	AF	11
489	Rainbow Canyon	D	AF	11
490	Rainbow Canyon	D	AF	11
491	Rainbow Canyon	D	AF	11
377	River Mts.	D	AH	57
396	Indian Ridge	D	AN	12
401	Arrow Canyon Range	D	AN	12
438	Meadow Valley Mts.	D	AN	12
440	Meadow Valley Mts.	D	AN	12
443	Delamar Mts.	D	AN	12
482	River Mts.	D	AN	12
260	Virgin Mts., V-1	D	AP	13

Appendix D (continued)

PCU #	Locality	Cyt *b* haplotype	Control region haplotype	both
285	Virgin Mts., V-1	D	AP	13
287	Virgin Mts., V-1	D	AP	13
286	Virgin Mts., V-1	D	AQ	14
290	Virgin Mts., V-1	D	AQ	14
402	Arrow Canyon Range	D	B	15
406	Arrow Canyon Range	D	B	15
403	Arrow Canyon Range	D	C	16
405	Arrow Canyon Range	D	D	17
433	East Mormon Mts.	D	L	18
455	Mormon Mts.	D	L	18
456	Mormon Mts.	D	L	18
434	East Mormon Mts.	D	M	19
480	Mt. Irish Range	D	N	20
481	Mt. Irish Range	D	N	20
359	Muddy Mts.	D	R	21
363	Muddy Mts.	D	R	21
364	Muddy Mts.	D	R	21
397	Indian Ridge	D	S	22
439	Meadow Valley Mts.	D	Z	23
432	East Mormon Mts.	D		
485	River Mts.	D		
332	Eldorado Mts.	E	I	24
334	Eldorado Mts.	E	K	25
338	McCullough Mts.	E	V	26
339	McCullough Mts.	E	V	26
341	McCullough Mts.	E	W	27
342	McCullough Mts.	E	W	27
330	Eldorado Mts.	F	G	28
333	Eldorado Mts.	F	G	28
331	Eldorado Mts.	G	H	29
335	Eldorado Mts.	H	J	30
479	Mt. Irish Range	I	N	31
391	Specter Range	J	AJ	32
346	Goodsprings	K	P	33
349	Goodsprings	K	P	33
347	Goodsprings	K	Q	34
281	Newberry Mts., NB-3	L	BD	35

PCU #	Locality	Cyt *b* haplotype	Control region haplotype	both
343	Goodsprings	L	P	36
348	Goodsprings	L	Q	37
350	Goodsprings	L	Q	37
398	Indian Ridge	M	AM	38
399	Indian Ridge	M	AM	38
392	Specter Range	N	AL	39
394	Specter Range	N	AL	39
352	Last Chance Range	N	AM	40
353	Last Chance Range	N	AM	40
355	Last Chance Range	N	AM	40
356	Last Chance Range	N	AM	40
357	Last Chance Range	N	AM	40
389	Specter Range	N	AM	40
393	Specter Range	N	AM	40
354	Last Chance Range	N	T	41
388	Specter Range	N		
475	Black Marble Mtn.	N		
340	McCullough Mts.	O	Y	42
237	Meadow Valley Mts.	P	X	43
271	Newberry Mts., NB-1	Q	BG	44
264	Newberry Mts., NB-3	R	AE	45
288	Newberry Mts., NB-3	R	AE	45
289	Newberry Mts., NB-3	R	AE	45
280	Newberry Mts., NB-3	S	BE	46
267	Newberry Mts., NB-3	T	BC	47
376	River Mts.	U	AG	48
483	River Mts.	U	AI	49
484	River Mts.	U	AN	50
486	Sheep Range	V	U	51
395	Specter Range	W	AK	52
410	Spotted Range	W	AM	53
411	Spotted Range	W	AM	53
460	Spotted Range	W	AM	53
462	Spotted Range	W	AM	53
461	Spotted Range	X	AM	54
227	Tikaboo Valley	Y	AN	55
404	Arrow Canyon Range		AN	

Appendix D (continued)

PCU #	Locality	Cyt *b* haplotype	Control region haplotype	both
293	Virgin Mts., V-1		AQ	
294	Virgin Mts., V-1		AQ	
295	Newberry Mts., NB-3		BB	
297	Newberry Mts., NB-3		BD	
296	Newberry Mts., NB-3		BF	
226	Newberry Mts.		BG	

Appendix E

Laboratory protocols for genetic analysis.

Tissue was taken mostly from liver, some muscle, and toe clips and stored either at –80 degrees Celsius (˚C) or in 95% ethanol. DNA was extracted by powdering approximately 100 milligrams (mg) of tissue in a prechilled mortar and pestle under liquid nitrogen. The resulting powder (around 100 mg) was transferred to an Eppendorf tube and mixed with 750 microliters (µl) of 1X STE buffer [100 millimolar (mM) NaCl + 10 mM Tris + 1 mM Ethylenediameinetetraacetic acid or EDTA, pH 7.5]. This mixture was then lysed by adding 10 µl of a 10 mg/milliliter(ml) stock solution of proteinase K, 20 µl 20% SDS (20 grams (g) lauryl sulfate sodium salt/100 ml distilled H_20), and incubated for three hours (h) at 55˚C. Lysis was followed by extraction. Extraction was accomplished by adding an equal volume of commercial grade phenol/chloroform/isoamyl alcohol (PCI) solution (equal volumes at the proportion of 25:24:1) and lightly shaken for five minutes (min) and then spun down for five min at 7,000 cycles/min. The supernatant liquid above the PCI was collected and PCI extraction was performed again. The resulting product from the second extraction was followed by a final washing of chloroform/isoamyl alcohol (equal volumes of 24:1) shaken for five min, then spun down at 7,000 cycles/min. The DNA was then precipitated out of solution with 1.0 ml of 100% ethanol and 50 µl 2M NaCl for eight h at –20˚C. This was spun down for 20 min at 11,000 cycles/min. The ethanol was decanted (taking care not to lose the DNA pellet) and allowed to dry for 40 min at 55˚C. This was resuspended in 1X TE buffer (1 mM EDTA + 10 mM Tris, pH 8.0) for one h at 65˚C. Lanes in a 1% agarose 1X Trisbase, boric acid, EDTA (TBE) gel were loaded with 5 µl of extraction product and 2 µl of marker dye and electrophoresed at 105 volts for approximately 40 min. Gel was stained in ethidium bromide for ten min and destained for 15 min in H_20. Images of gels were recorded by illuminating the gel with an ultra-violet light table and photographed with a glass lens video camera fed into image capturing software (Alpha Ease™ version 3.25, 1996). DNA concentration was determined by either estimating product compared to a lane of 5 µl of 100 nanograms (ng) calf thymus DNA marker in the gel, or by quantifying with a fluorometer (DyNA Quant™ 200, 1995). DNA was stored in a –20˚C freezer.

Approximately 0.1 ng of DNA was used to amplify a 428 base pair (bp) section of cytochrome *b* and a 528 bp region of the control region. Primers used for PCR amplification and sequence reactions were L15783 (Macy et al., 1997): 5'-CAA CCA GTA GAA GAC CC-3', and Hdloopchuck (this study): 5'-GTC CGA TAA CTT AGT CTA ACA ATC A-3' for the control region fragment and primers I designed, Chuckcytb-F: 5'-GTA ATG GCC ACA GCA TTC GTA GGC T-3' and Chuckcytb-R: 5'-ATT GAG AAG AGT AGG GCR AGT AC-3', for the cytochrome *b* fragment. Primers were purchased from Integrated DNA Technologies Inc. (IDT®). Amplifications for PCR were done in 50- or 100-µl reactions using 1% Taq DNA polymerase, 10% buffer, and 6% MgCl (stock concentrations, as per Promega kit, catalog No. M1661), 16% dNTP's (100 µM), 2.5% of each primer (10µM), and 0.5–1% of DNA template depending on concentration. PCRs were performed in a Perkin-Elmer GeneAmp® thermocycler (PCR System 2400, version 2.10, 1996; and PCR System 9700, version 2.25, 1997) under the following two profiles: for cytochrome *b*, hot start at 94˚C for five min, 30 cycles of

denature at 94˚C for one min and 30 seconds (sec), annealing at 42˚C for two min, elongation at 72˚C for three min, and a final elongation at 72˚C for ten min and held at 4˚C until removal; for control region, hot start at 94˚C for five min, 30 cycles of denature at 94˚C for 35 sec, annealing at 45˚C for 35 sec, elongation at 70˚C for two min and 30 sec, and a final elongation at 70˚C for ten minutes and held at 4˚C until removal.

PCR products were checked on a 1% agarose gel in TBE, and stained with ethidium bromide. Fragment size was confirmed by comparing to a manufactured ladder of known fragment lengths (Bioline 5µl Hyperladder; Bioline). Both products were confirmed to be from the proper region in the gene by aligning the fragments with identified regions available on GenBank. Each primer for cytochrome *b* and control region amplifies approximately 500- and 710-bp fragments, respectively, and each gene has a primer from the 5' and the complimentary 3' direction. Therefore, no internal primers were needed and all nucleotides were confirmed by sequences from both directions. PCR products were purified for sequencing PCR reactions using a Vac-Man™ Laboratory Vacuum manifold used in conjunction with Wizard™ DNA Purification resin and direct purification buffer. Sequence reaction profile was modified by reducing the volume of Big Dye ® Terminator Ready Reaction Mix to one quarter of the manufacturer's recommended amount. The following reagents were added to separate tubes for each reaction: 2 µl Big Dye® Terminator Ready Reaction Mix, 1.5 µl Sequencing Buffer (400 mM Tris-HCl + 10 mM $MgCl_2$, pH 9.0), 2 µl primer (1.6 µM), 3–10 ng cytochrome *b* DNA template or 5–20 ng control region DNA template, topped off with the necessary amount of double distilled H_20 for a total reaction volume of 10 µl. These tubes were then placed in a PCR System 9700 and the following protocol was repeated for 30 cycles: rapid thermal ramp (1˚C/sec) to 96˚C, 96˚C for ten sec, rapid thermal ramp to 50˚C, 50˚C for five sec, rapid thermal ramp to 60˚C, 60˚C for three min, rapid thermal ramp to 4˚C and held until ready to purify. Contents of tubes were then spun down in a microcentrifuge. Prior to sequencing, this product was purified (removal of dye terminators and other low molecular weight components) using the manufacturer's Centri-SEP protocol (Princeton Separations, version 5.0, 1995).

Sequencing was done using a Perkin-Elmer 1998 ABI Prism® 377 automated DNA sequencer and analyzed with DNA Sequencing Analysis Software version 3.3, 1998. Protocols for sequencing followed the manufacturer's recommendations (ABI Prism 377 DNA Sequencer for sequencing and Gene Scan Software Application User's Manual, 1998, Perkin-Elmer Corporation). Sequence comparisons and alignment were done using Sequencher 3.1 and PAUP*4.0b2 (Swofford, 1999).

Appendix F

Raw data for fragment analyses

Sequence Variation for cytochrome *b* Fragment

Of the 105 individuals assayed 28 unique haplotypes were recovered from cytochrome *b* fragment. This fragment consisted of 428 characters with no gaps. There were 38 variable characters, 27 of which were parsimony informative. Even with this little variation the distribution of 10,000 trees generated randomly from the cytochrome *b* data set was significantly left skewed (g_1 = -1.127485, $t_{.001}$ = -9.5226291, $df = \infty$, $P <$ 0.001; mean ± standard deviation tree length = 174.0606 ± 11.4, range = 103–192), strongly suggesting the presence of phylogenetic signal in the data.

Sequence Variation for Control Region Fragment

Of the 105 individuals assayed 46 unique haplotypes were recovered from the control region fragment. This fragment consisted of 582 characters with one gap. There were 59 variable characters, 32 of which were parsimony informative. The distribution of 10,000 trees generated randomly from the control region data set was significantly left skewed (g_1 = -0.415976, $t_{.001}$ = -4.096, $df = \infty$, $P < 0.001$; mean ± standard deviation tree length = 286.82 ± 10.2, range = 232–317) revealing the appropriateness of phylogenetic analysis.

TCS Analysis for the cytochrome *b* Fragment

The nesting design that resulted from the analysis of cytochrome *b* haplotypes indicates that 24 haplotypes (I–XIV, XVI–XXIII, XXV, and XXVI) appear at the tips of the cladogram and that haplotypes XV and XXIV are interior, or ancestral (Figure 15). Together, these 26 haplotypes are arranged in nine one-step clades, four two-step clades, and two three-step clades (Figure 16). The two three step clades are connected by a minimum of 17 mutational steps, well beyond the confidence limits of parsimony. Remember that this analysis does not resolve differences at this level with much confidence. Consequently, the relationship between the two is represented by the clades inferred in the previous chapter. As an aid in interpreting the results, Figure 17 presents a rough overlay of the cladogram over geography. The interior clade 1-4, consists of haplotype XV that is fixed in nine localities (7–14 and 22) and six haplotypes (IX–XIV) from six localities (3, 5, 6, 15, 23, 25). This clade is separated from 1-5 by one mutational step to form Clade 2-2. Clade 2-2 covers a geographic area of contiguous mountain ranges roughly connected by the Muddy/Virgin River drainages. Clade 2-3 is comprised of five haplotypes (XVII–XXI) from two mountain ranges (16 and 17). Clade 2-1 is comprised of eight haplotypes from eight localities that are found outside and west of the Muddy/Virgin drainages (except for the Hiko Range). Clade 2-4 is comprised of five haplotypes (XXII–XXVI) from two localities (Newberry Mountains and Goodsprings). Clades 2-1, 2-2, and 2-3 are all separated by one mutational step to form clade 3-1.

Homogeneity testing revealed significant nonrandom association of clades and sampling locations, indicating phylogeographic structure in the data at higher clade levels (Table 4). Table 5 presents the results of the nested cladistic analysis of geographical distance for the cytochrome *b* data set. Table 6 presents the results obtained when the

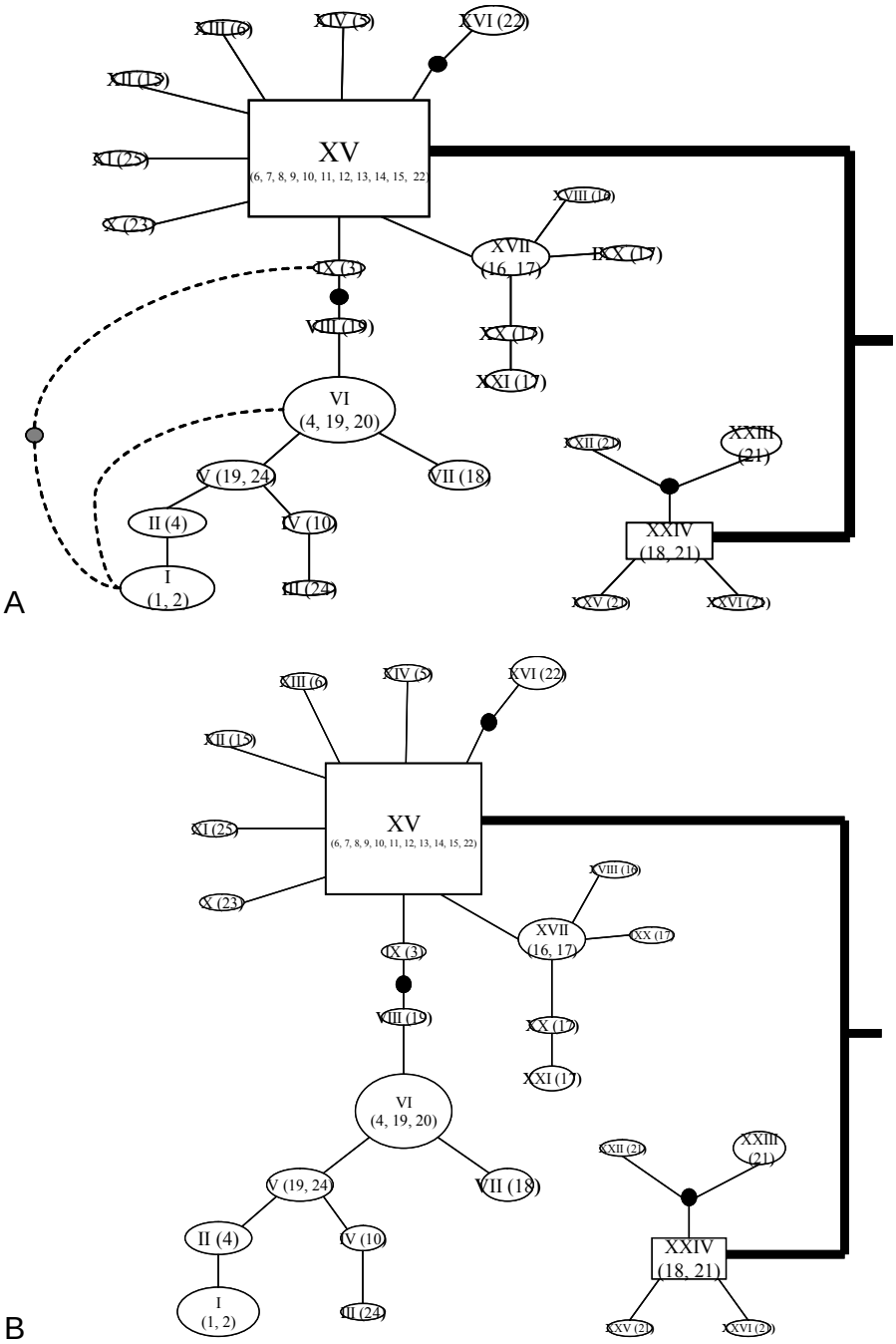

Figure 15. A) The haplotype network for cytochrome *b* haplotypes as estimated for *Sauromalus obesus.* B) The unambiguous haplotype network eliminating ambiguous connections after following the rules given in Templeton and Sing (1992). Each line in the network represents a single, unambiguous mutational change. Black dots indicate an interior node in the network that was not present in the sample; that is, these are inferred intermediate haplotypes between two nearest-neighbor haplotypes in the network that differed by two or more mutations. Dashed lines and gray dots indicate ambiguous but equally likely connections, using the criteria in Templeton et al., (1992). Size of haplotype circles and squares is roughly proportional to haplotype frequency. Squares represent haplotypes with the greatest outgroup probability. Roman numerals represent unique haplotypes and Arabic numerals are the localities where the unique haplotypes are found. Locality numbers are listed and mapped in Figure 7.

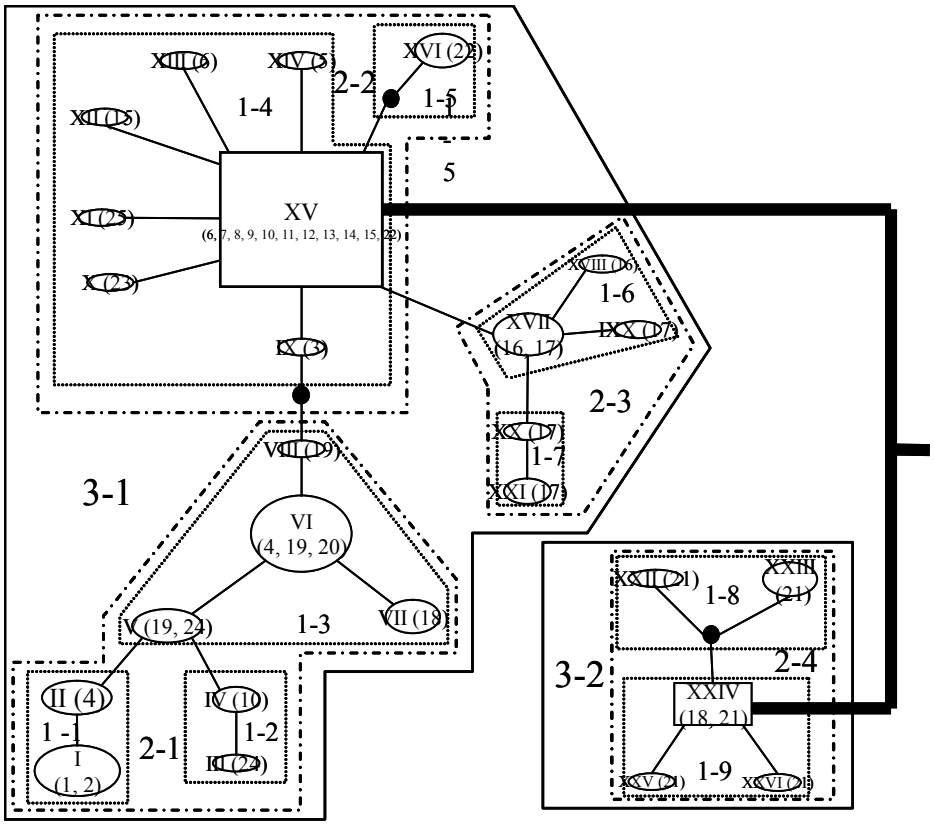

Figure 16. The haplotype network for cytochrome *b* haplotypes as estimated for *Sauromalus obesus* with the nesting design of Templeton et al. (1987). Each line in the network represents a single, unambiguous mutational change. Black dots indicate an interior node in the network that was not present in the sample; that is, these are inferred intermediate haplotypes between two nearest neighbor haplotypes in the network that differed by two or more mutations. Size of haplotype circles and squares is roughly proportional to haplotype frequency. Squares represent haplotypes with the greatest outgroup probability. Roman numerals represent unique haplotypes and Arabic numerals in parentheses are the localities where the unique haplotypes are found. Arabic numerals separated by dashes indicate nested clade level and are separated by different outline patterns. Locality numbers are listed and mapped in Figure 7.

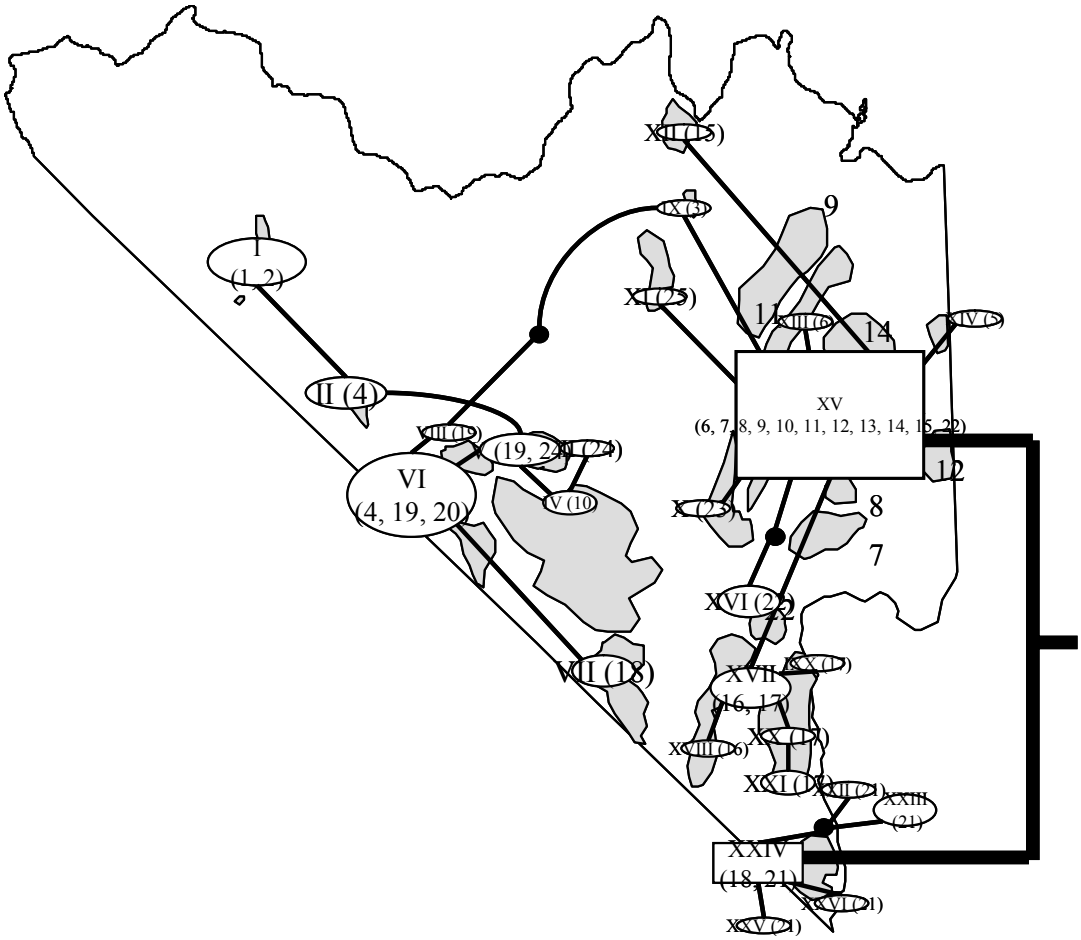

Figure 17. The haplotype network for cytochrome *b* haplotypes as estimated for *Sauromalus obesus* overlaid on their geographic location. Each line in the network represents a single, unambiguous mutational change. Black dots indicate an interior node in the network that was not present in the sample; that is, these are inferred intermediate haplotypes between two nearest neighbor haplotypes in the network that differed by two or more mutations. Size of haplotype circles and squares is roughly proportional to haplotype frequency. Squares represent haplotypes with the greatest outgroup probability. Roman numerals represent unique haplotypes and Arabic numerals are the localities where the unique haplotypes are found. Locality numbers are listed in Figure 7.

Table 4. Nested contingency analysis of geographical associations for cytochrome *b* haplotypes.

Clade	Permutational chi-square statistic	Probability
1-1	13.0	0.001
1-2	3.0	0.332
1-3	38.9	0.001
1-4	215.5	< 0.001
1-6	2.3	0.655
1-9	3.0	0.392
2-1	58.9	< 0.001
2-2	22.9	0.108
2-3	3.4	0.170
2-4	2.9	0.221
3-1	189.4	< 0.001
Entire Cladogram	86.7	< 0.001

Table 5. Results of the nested geographical analysis of *Sauromalus obesus* cytochrome *b* fragment

Clade	Nested Clades	Position	Type of Distance	Distance	Probability <=	Probability >=
1-1	I	tip	D_c	15.37	0.00	1.00
			D_n	25.37	0.00	1.00
	II	interior	D_c	0.00	0.00	1.00
			D_n	53.17	1.00	0.00
			$D_cI - D_cT$	-15.37	0.17	0.83
			$D_nI - D_nT$	27.80	1.00	0.00
1-2	III	tip	D_c	0.00	1.00	1.00
			D_n	15.78	1.00	0.33
	IV	interior	D_c	0.00	0.33	1.00
			D_n	6.31	0.00	1.00
			$D_cI - D_cT$	0.00	0.33	1.00
			$D_nI - D_nT$	-9.46	0.00	1.00
1-3	V	tip	D_c	10.19	0.00	1.00
			D_n	28.70	0.22	0.78
	VI	interior	D_c	26.25	0.02	0.98
			D_n	29.54	0.02	0.98
	VII	tip	D_c	0.00	0.03	1.00
			D_n	79.70	1.00	0.00
	VIII	tip	D_c	0.00	0.72	1.00
			D_n	34.25	0.81	0.19
			$D_cI - D_cT$	20.59	0.91	0.09
			$D_nI - D_nT$	-16.77	0.03	0.97
1-4	IX	tip	D_c	0.00	0.85	1.00
			D_n	74.34	0.74	0.26
	X	tip	D_c	0.00	0.86	1.00
			D_n	63.88	0.65	0.37
	XI	tip	D_c	0.00	0.87	1.00
			D_n	74.83	0.82	0.20
	XII	tip	D_c	0.00	0.90	1.00
			D_n	97.04	1.00	0.00

Table 5 (continued)

Clade	Nested Clades	Position	Type of Distance	Distance	Probability <=	Probability >=
1-4	XIII	tip	D_c	0.00	0.05	1.00
			D_n	16.61	0.01	1.00
	XIV	tip	D_c	0.00	0.88	1.00
			D_n	78.41	0.84	0.17
	XV	interior	D_c	51.63	0.00	1.00
			D_n	53.16	0.00	1.00
			$D_cI - D_cT$	51.63	0.65	0.35
			$D_nI - D_nT$	-7.08	0.15	0.85
1-6	XVII	interior	D_c	13.02	0.11	0.89
			D_n	13.53	0.66	0.89
	XVIII	tip	D_c	0.00	1.00	1.00
			D_n	10.45	0.64	1.00
	IXX	tip	D_c	0.00	1.00	1.00
			D_n	20.91	1.00	0.40
			$D_cI - D_cT$	13.02	0.11	0.89
			$D_nI - D_nT$	-2.15	0.66	0.89
1-9	XXIV	interior	D_c	35.24	0.00	1.00
			D_n	48.80	0.00	1.00
	XXV	tip	D_c	0.00	0.48	1.00
			D_n	54.98	0.48	0.52
	XXVI	tip	D_c	0.00	0.53	1.00
			D_n	54.98	0.53	0.47
			$D_cI - D_cT$	35.24	0.19	0.81
			$D_nI - D_nT$	-6.19	0.19	0.81
2-1	1-1	tip	D_c	33.55	0.00	1.00
			D_n	73.03	0.93	0.07
	1-2	tip	D_c	9.02	0.01	0.99
			D_n	66.39	0.60	0.40
	1-3	interior	D_c	36.74	0.00	1.00
			D_n	51.59	0.03	0.97
			$D_cI - D_cT$	7.79	0.65	0.35
			$D_nI - D_nT$	-20.19	0.04	0.96

Table 5 (continued)

Clade	Nested Clades	Position	Type of Distance	Distance	Probability <=	Probability >=
2-2	1-4	interior	D_c	58.79	0.17	0.83
			D_n	58.90	0.18	0.82
	1-5	tip	D_c	0.00	0.00	1.00
			D_n	84.68	0.97	0.03
			$D_cI - D_cT$	58.79	1.00	0.01
			$D_nI - D_nT$	-25.78	0.03	0.97
2-3	1-6	interior	D_c	13.94	0.17	0.94
			D_n	15.68	0.11	0.89
	1-7	tip	D_c	0.00	0.17	1.00
			D_n	15.69	1.00	0.00
			$D_cI - D_cT$	13.94	1.00	0.11
			$D_nI - D_nT$	0.00	0.11	0.89
2-4	1-8	interior	D_c	0.00	0.00	1.00
			D_n	34.05	0.00	1.00
	1-9	tip	D_c	50.70	0.82	0.19
			D_n	52.31	0.82	0.19
			$D_cI - D_cT$	-50.70	0.00	1.00
			$D_nI - D_nT$	-18.26	0.00	1.00
3-1	2-1	tip	D_c	61.75	0.00	1.00
			D_n	112.24	1.00	0.00
	2-2	interior	D_c	59.79	0.00	1.00
			D_n	75.42	0.00	1.00
	2-3	tip	D_c	15.68	0.00	1.00
			D_n	113.92	0.95	0.05
			$D_cI - D_cT$	8.60	0.76	0.24
			$D_nI - D_nT$	-37.21	0.00	1.00
Total	3-1	interior	D_c	90.85	0.04	0.96
			D_n	90.65	0.02	0.99
	3-2	interior	D_c	45.36	0.01	0.99
			D_n	145.27	1.00	0.00

Table 6. Inference chain for cytochrome b data based on results of geographic dispersion analysis given in Table 5.

Clade	Chain of Inference	Inference
Haplotypes nested in 1-1	1, 2a, 3, 5, 6, 7, 8	Sampling Inadequate to Discriminate between Isolation by Distance versus Long Distance Dispersal
Haplotypes nested in 1-2	1, 2, 11, 17	Inconclusive outcome
Haplotypes nested in 1-3	1, 2a, 3, 5, 6, 7	Restricted Gene Flow/Dispersal but with some Long Distance Dispersal
Haplotypes nested in 1-4	1, 2, 11b, 12	Contiguous Range Expansion
Haplotypes nested in 1-9	1, 2, 11, 12	Contiguous Range Expansion
One-step clades nested in 2-1	1, 11, 12	Contiguous Range Expansion
One-step clades nested in 2-2	1, 2a, 3, 5, 6, 7	Restricted Gene Flow/Dispersal but with some Long Distance Dispersal
One-step clades nested in 2-3	1, 2, 11, 17	Inconclusive outcome
One-step clades nested in 2-4	1, 2, 11, 12	Contiguous Range Expansion
Two-step clades nested in 3-1	1, 2, 11, 12	Contiguous Range Expansion
Three-step clades nested in the entire cladogram	17 mutational steps between 3-1 and 3-2	Contiguous Range Expansion

Inference key is found in Appendix K

inference key given in the GEODIS web site (Templeton, et al 1995) is applied to the statistical results given in Table 5 along with resulting inferences about population structure and history. Geographic distributions of clades indicate two well-supported population fragmentation events: Restricted gene flow/dispersal but with some long distance dispersal and contiguous range expansion (Table 6). Restricted gene flow is found in different extents among haplotypes nested in Clades 1-1 and 1-3 and among clades nested in Clade 2-2. Contiguous range expansion is found among haplotypes nested in Clades 1-4 and 1-9 and among clades nested in Clades 2-1, 2-4, 3-1, and the entire cladogram.

TCS for the Control Region Fragment

The nesting design that resulted from the analysis of the control region haplotypes indicates that 39 haplotypes appear at the tips and that haplotype XVIII is interior, or ancestral (Figure 18). Together, these 40 haplotypes are arranged in 19 one-step clades, ten two-step clades, seven three-step clades, five four-step clades, three five-step clades, and two six-step clades (Figure 19). The two six step clades are connected by a minimum of seven mutational steps, well beyond the confidence limits of parsimony. This analysis does not resolve differences at this level with much confidence. Consequently, the relationship between the two is represented by the clades inferred in the previous chapter. As an aid in interpreting the results, Figure 20 presents a rough overlay of the cladogram over geography. The interior clade 1-6 is comprised of the most

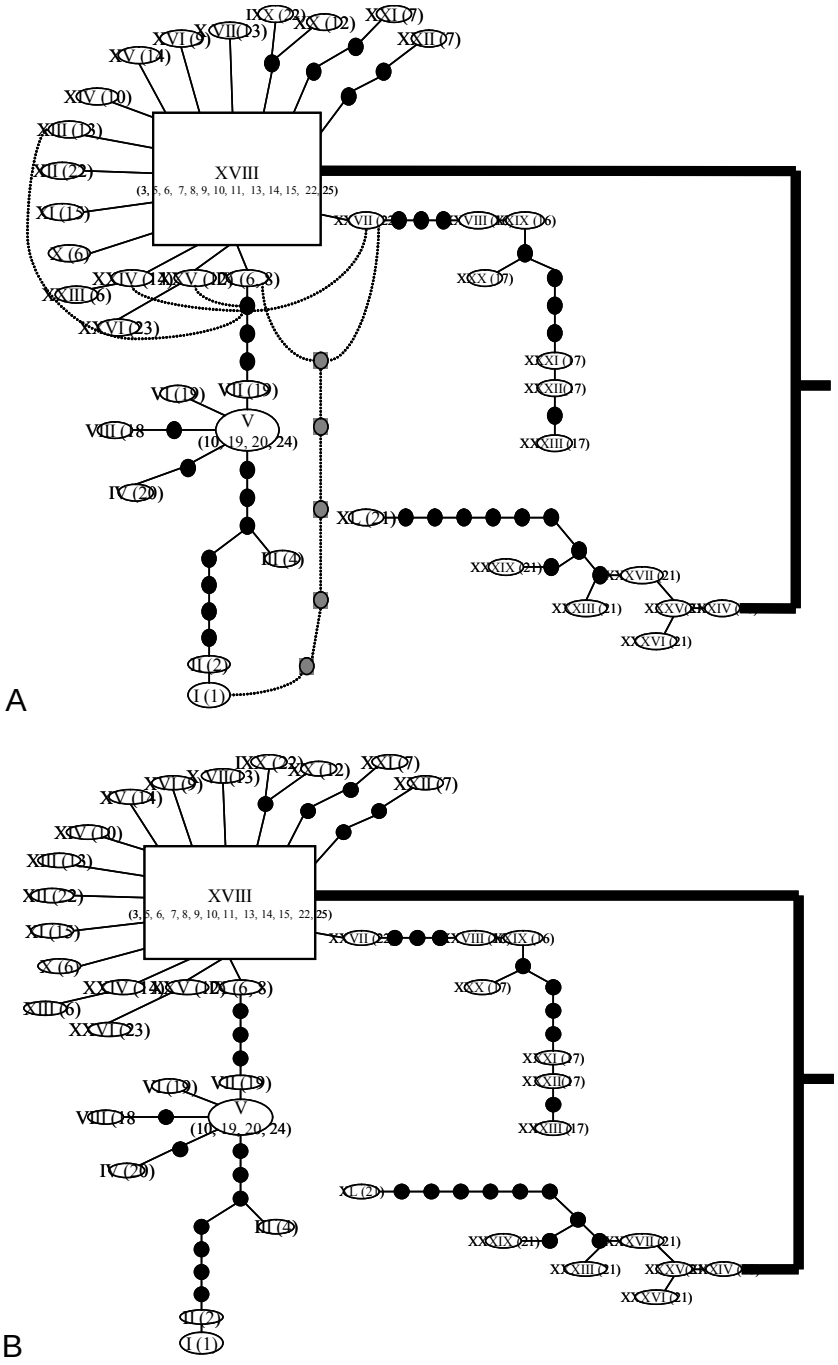

Figure 18. A) The haplotype network for control region haplotypes as estimated for *Sauromalus obesus.* B) The unambiguous haplotype network for control region haplotypes eliminating ambiguous connections after following the rules given in Templeton and Sing (1992). Each line in the network represents a single, unambiguous mutational change. Black dots indicate an interior node in the network that was not present in the sample; that is, these are inferred intermediate haplotypes between two nearest neighbor haplotypes in the network that differed by two or more mutations. Dashed lines and gray dots indicate ambiguous but equally likely connections, using the criteria in Templeton et al., (1992). Size of haplotype circles and squares is roughly proportional to haplotype frequency. Squares represent haplotypes with the greatest outgroup probability. Roman numerals represent unique haplotypes and Arabic numerals are the localities where the unique haplotypes are found. Locality numbers are listed and mapped in Figure 7.

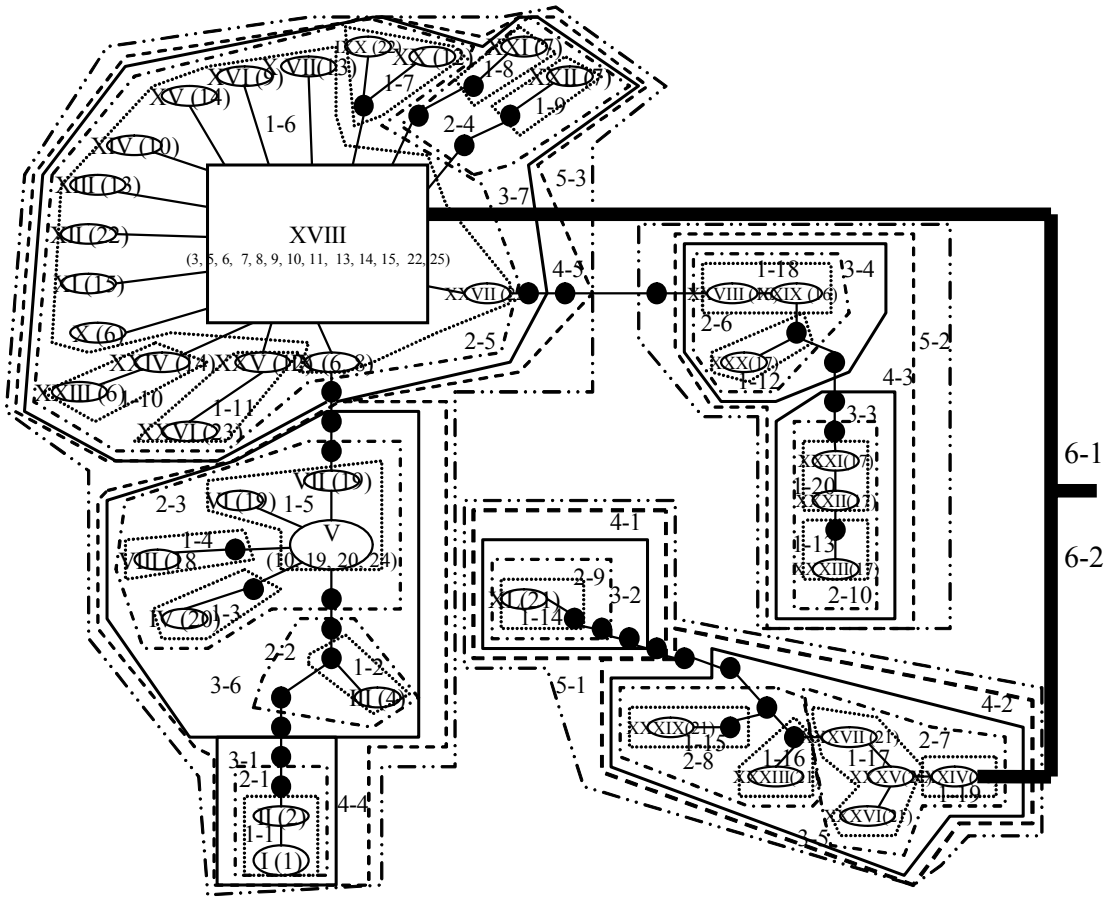

Figure 19. The haplotype network for control region haplotypes as estimated for *Sauromalus obesus* with the nesting design of Templeton et al., (1987). Each line in the network represents a single, unambiguous mutational change. Black dots indicate an interior node in the network that was not present in the sample; that is, these are inferred intermediate haplotypes between two nearest neighbor haplotypes in the network that differed by two or more mutations. Size of haplotype circles and squares is roughly proportional to haplotype frequency. Squares represent haplotypes with the greatest outgroup probability. Roman numerals represent unique haplotypes and Arabic numerals in parentheses are the localities where the unique haplotypes are found. Arabic numerals separated by dashes indicate nested clade level and are separated by different outline patterns. Locality numbers are listed and mapped in Figure 7.

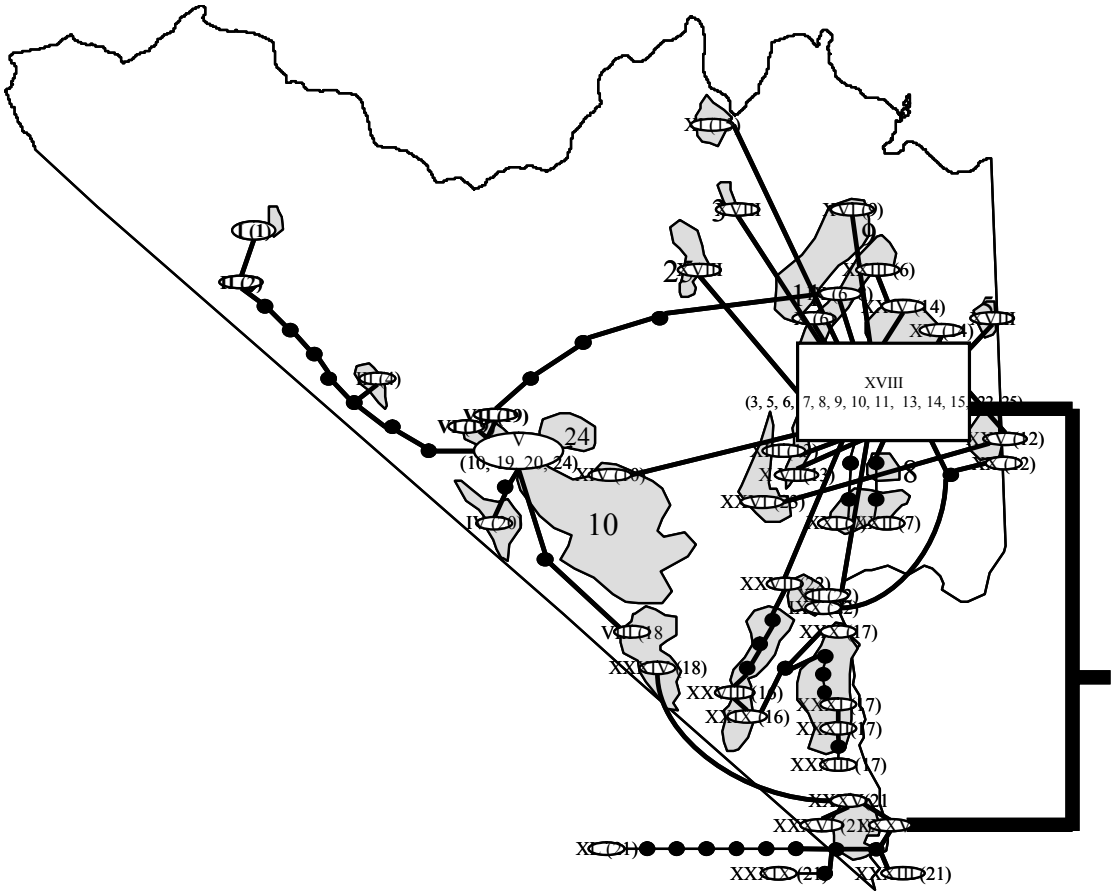

Figure 20. The haplotype network for control region haplotypes as estimated for *Sauromalus obesus* overlaid on their geographic location. Each line in the network represents a single, unambiguous mutational change. Black dots indicate an interior node in the network that was not present in the sample; that is, these are inferred intermediate haplotypes between two nearest neighbor haplotypes in the network that differed by two or more mutations. Size of haplotype circles and squares is roughly proportional to haplotype frequency. Squares represent haplotypes with the greatest outgroup probability. Roman numerals represent unique haplotypes and Arabic numerals are the localities where the unique haplotypes are found. Locality numbers are listed in Figure 7.

common and most geographically widespread interior haplotype XVIII (fixed in thirteen localities [3, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 22, 25]). This clade is joined with geographically and cladistically proximate clades to form Clade 4-5. Clade 4-5 covers a geographic area of contiguous mountain ranges roughly connected by the Muddy/Virgin River drainages. Haplotypes in tip Clades 4-3 and 4-4 are separated from haplotypes in interior Clade 4-5 by three mutations. Clade 4-3 is made of six haplotypes from two localities (16, McCullough Range and 17, Eldorado Mountains). Clade 4-4 is made of eight haplotypes from eight localities (1, 2, 4, 10, 18, 19, 20, and 24) that are found outside of the Muddy/Virgin River drainages. Clade 5-1 is made of seven haplotypes from two localities (18, Goodsprings and 21, Newberry Mountains).

Homogeneity testing revealed significant nonrandom association of clades and sampling locations, indicating phylogeographic structure in the data at higher clade levels (Table 7). Table 8 presents the results of the nested cladistic analysis of geographical distance for the control region data set. Table 9 presents the results obtained when the inference key given in the GEODIS web site (Templeton, et al 1995) is applied to the statistical results given in Table 8 along with resulting inferences about population structure and history. Geographic distributions of clades indicate four well-supported population fragmentation events: restricted gene flow with isolation by distance, restricted gene flow/dispersal but with some long distance dispersal, past fragmentation, and contiguous range expansion (Table 9). Restricted gene flow is found in different extents among haplotypes nested in Clades 1-1 and 1-6 and among clades nested in Clade 2-5, 3-5, 3-6, 5-1, 6-1, and the entire cladogram. Contiguous range expansion is found among clades nested in Clades 2-6, 3-7, 4-3, 4-4, and 5-3. Past fragmentation is found among clades nested in Clades 2-3 and 2-7.

Table 7. Nested contingency analysis of geographical associations for control region haplotypes.

Clade	Permutational chi-square statistic	Probability
1-1	8.0	0.025
1-5	7.2	0.318
1-6	156.4	0.067
1-7	4.0	0.260
1-10	4.0	0.228
1-11	6.0	0.149
2-3	24.6	0.002
2-5	68.5	0.010
2-6	8.0	0.027
2-7	9.0	0.013
3-5	1.7	0.514
3-6	27.0	< 0.001
3-7	27.9	0.084
4-3	1.5	0.500
4-4	34.0	< 0.001
5-1	0.3	1.000
5-3	82.8	< 0.001
6-1	98.0	< 0.001
Entire Cladogram	100.0	< 0.001

Table 8. Results of the nested geographical analysis of *Sauromalus obesus* control region fragment haplotypes.

Clade	Nested Clades	Position	Type of Distance	Distance	Probability <=	Probability >=
1-1	I	tip	D_c	0.00	0.00	1.00
			D_n	15.37	0.00	1.00
	II	interior	D_c	0.00	0.02	1.00
			D_n	15.37	0.98	0.02
			$D_cI - D_cT$	0.00	1.00	0.00
			$D_nI - D_nT$	0.00	0.98	0.02
1-5	V	interior	D_c	26.14	0.33	0.69
			D_n	26.66	0.35	0.66
	VI	tip	D_c	0.00	0.70	1.00
			D_n	29.76	0.61	0.39
	VII	tip	D_c	0.00	0.18	1.00
			D_n	29.76	0.58	0.42
			$D_cI - D_cT$	26.14	0.86	0.16
			$D_nI - D_nT$	-3.10	0.34	0.66
1-6	XVIII	interior	D_c	55.69	0.46	0.54
			D_n	55.63	0.41	0.59
	IX	tip	D_c	19.61	0.26	0.75
			D_n	56.36	0.41	0.59
	X	tip	D_c	0.00	0.83	1.00
			D_n	3.85	0.12	0.88
	XI	tip	D_c	0.00	0.00	1.00
			D_n	84.12	0.97	0.04
	XII	tip	D_c	0.00	0.82	1.00
			D_n	98.59	1.00	0.12
	XIII	tip	D_c	0.00	0.06	1.00
			D_n	50.07	0.26	0.76
	XIV	tip	D_c	0.00	0.82	1.00
			D_n	87.79	0.87	0.18
	XV	tip	D_c	0.00	0.82	1.00
			D_n	45.42	0.20	0.80
	XVI	tip	D_c	0.00	0.00	1.00
			D_n	50.21	0.27	0.73

Table 8 (continued)

Clade	Nested Clades	Position	Type of Distance	Distance	Probability <=	Probability >=
1-6	XVII	tip	D_c	0.00	0.82	1.00
			D_n	50.07	0.38	0.77
			$D_cI - D_cT$	53.38	1.00	0.00
			$D_nI - D_nT$	-3.31	0.43	0.57
1-7	IXX	tip	D_c	0.00	1.00	1.00
			D_n	58.86	1.00	0.27
	XX	tip	D_c	0.00	0.27	1.00
			D_n	22.85	0.27	1.00
1-10	XXIII	tip	D_c	0.00	1.00	1.00
			D_n	35.13	1.00	0.26
	XXIV	interior	D_c	0.00	0.26	1.00
			D_n	11.72	0.26	1.00
			$D_cI - D_cT$	0.00	0.26	1.00
			$D_nI - D_nT$	-23.41	0.26	1.00
1-11	XXV	interior	D_c	0.00	0.17	1.00
			D_n	42.89	1.00	0.17
	XXVI	tip	D_c	0.00	1.00	1.00
			D_n	30.68	0.17	1.00
			$D_cI - D_cT$	0.00	0.17	1.00
			$D_nI - D_nT$	12.22	1.00	0.17
2-3	1-3	tip	D_c	0.00	0.72	1.00
			D_n	24.79	0.51	0.77
	1-4	tip	D_c	0.00	0.02	1.00
			D_n	74.66	1.00	0.00
	1-5	interior	D_c	27.08	0.00	1.00
			D_n	29.56	0.00	1.00
			$D_cI - D_cT$	27.08	0.96	0.05
			$D_nI - D_nT$	-32.63	0.00	1.00
2-5	1-6	interior	D_c	57.89	0.36	0.65
			D_n	60.38	0.61	0.39
	1-7	tip	D_c	32.93	0.21	0.79

Table 8 (continued)

Clade	Nested Clades	Position	Type of Distance	Distance	Probability <=	Probability >=
2-5	1-7	tip	D_n	73.04	0.86	0.14
	1-10	tip	D_c	17.57	0.02	0.98
			D_n	32.24	0.04	0.96
	1-11	tip	D_c	35.77	0.13	0.88
			D_n	65.43	0.66	0.34
			$D_cI - D_cT$	28.13	0.97	0.03
			$D_nI - D_nT$	2.26	0.61	0.39
2-6	1-12	tip	D_c	0.00	0.20	1.00
			D_n	18.23	1.00	0.01
	1-18	interior	D_c	0.00	0.01	1.00
			D_n	13.13	0.01	0.99
			$D_cI - D_cT$	0.00	0.29	0.73
			$D_nI - D_nT$	-5.10	0.01	0.99
2-7	1-17	interior	D_c	0.00	0.00	1.00
			D_n	46.42	0.00	1.00
	1-19	tip	D_c	0.00	0.01	1.00
			D_n	55.59	1.00	0.01
			$D_cI - D_cT$	0.00	0.52	1.00
			$D_nI - D_nT$	-9.17	0.00	1.00
3-5	2-7	interior	D_c	50.59	0.72	0.28
			D_n	49.47	0.72	0.28
	2-8	tip	D_c	0.00	0.00	1.00
			D_n	34.05	0.00	1.00
			$D_cI - D_cT$	50.59	1.00	0.00
			$D_nI - D_nT$	15.41	1.00	0.00
3-6	2-2	tip	D_c	0.00	0.00	1.00
			D_n	58.20	0.97	0.03
	2-3	interior	D_c	35.20	0.01	0.99
			D_n	36.11	0.01	0.99
			$D_cI - D_cT$	35.20	1.00	0.00
			$D_nI - D_nT$	-22.10	0.02	0.98

Table 8 (continued)

Clade	Nested Clades	Position	Type of Distance	Distance	Probability <=	Probability >=
3-7	2-4	tip	D_c	59.92	0.62	0.39
			D_n	60.03	0.60	0.40
	2-5	interior	D_c	0.00	0.00	1.00
			D_n	53.74	0.34	0.66
			$D_cI - D_cT$	-59.92	0.00	1.00
			$D_nI - D_nT$	-6.29	0.34	0.66
4-3	3-3	tip	D_c	0.00	0.34	1.00
			D_n	9.74	0.34	0.66
	3-4	tip	D_c	15.27	1.00	0.00
			D_n	14.72	1.00	0.00
5-1	4-1	tip	D_c	0.00	0.21	1.00
			D_n	31.93	0.00	1.00
	4-2	interior	D_c	45.36	0.21	0.79
			D_n	44.65	0.21	0.79
			$D_cI - D_cT$	45.36	1.00	0.00
			$D_nI - D_nT$	12.72	1.00	0.00
5-2	4-3	tip	D_c	82.13	0.09	0.91
			D_n	114.39	1.00	0.00
	4-4	interior	D_c	59.78	0.00	1.00
			D_n	75.37	0.00	1.00
			$D_cI - D_cT$	-22.35	0.01	0.99
			$D_nI - D_nT$	-39.02	0.00	1.00
Total	5-1	interior	D_c	42.38	0.00	1.00
			D_n	146.73	1.00	0.00
	5-2	tip	D_c	90.22	0.04	0.96
			D_n	90.02	0.02	0.98
			$D_cI - D_cT$	-47.84	0.00	1.00
			$D_nI - D_nT$	56.71	1.00	0.00

Table 9. Inference chain for control region data based on results of geographic dispersion analysis given in Table 8.

Clade	Chain of Inference	Inference
Haplotypes nested in 1-1	1, 2c, 3, 5, 15, 16, 18	Geographic Sampling Scheme Inadequate to Discriminate Between Fragmentation, Range Expansion, and Isolation by Distance
Haplotypes nested in 1-5	1	Panmixia or small sample size
Haplotypes nested in 1-6	1, 2ac, 3, 5, 6, 7	Restricted Gene Flow/Dispersal but with some Long Distance Dispersal
Haplotypes nested in 1-7	1	Panmixia or small sample size
Haplotypes nested in 1-10	1	Panmixia or small sample size
Haplotypes nested in 1-11	1	Panmixia or small sample size
One-step clades nested in 2-3	1, 2a, 3, 5, 15	Past Fragmentation
One-step clades nested in 2-5	1, 2a, 3, 4	Restricted Gene Flow with Isolation by Distance
One-step clades nested in 2-6	1, 2, 11b, 12	Contiguous Range Expansion
One-step clades nested in 2-7	1, 2a, 3, 5, 15	Past Fragmentation
Two-step clades nested in 3-5	1, 2a, 3, 4	Restricted Gene Flow with Isolation by Distance
Two-step clades nested in 3-6	1, 2c, 3, 5, 6, 7	Restricted Gene Flow/Dispersal but with some Long Distance Dispersal
Two-step clades nested in 3-7	1, 2, 11bc, 12	Contiguous Range Expansion
Three-step clades nested in 4-3	1, 2, 11a, 12	Contiguous Range Expansion
Three-step clades nested in 4-4	1, 2, 11b, 12	Contiguous Range Expansion
Four-step clades nested in 5-1	1, 2c, 3, 4	Restricted Gene Flow with Isolation by Distance
Four-step clades nested in 5-3	1, 2, 11b, 12	Contiguous Range Expansion
Five-step clades nested in 6-1	1, 2a, 3, 5, 6, 7	Restricted Gene Flow/Dispersal but with some Long Distance Dispersal
Six-step clades nested in the entire cladogram	7 mutational steps between 6-1 and 6-2	Restricted Gene Flow/Dispersal but with some Long Distance Dispersal

Inference key is found in Appendix K

TCS for Both Gene Fragments

The nesting design that resulted from the analysis of combining the cytochrome *b* and control region fragments into unique haplotypes indicates that 53 haplotypes appear at the tips and that haplotype XLIII is interior, or ancestral (Figure 21). Together, these 54 haplotypes are arranged in 26 one-step clades, 13 two-step clades, nine three-step clades, seven four-step clades, four five-step clades, two six-step clades, and two seven-step clades (Figure 22). The two seven step clades are connected by a minimum of eight mutational steps, well beyond the confidence limits of parsimony. Since this analysis does not resolve differences at this level with much confidence, the relationship between the two is represented by the clades inferred in the previous chapter. As an aid in interpreting the results, Figure 23 presents a rough overlay of the cladogram over geography. The interior clade 1-10 is comprised of the most common and most geographically widespread interior haplotype XLIII (fixed in six localities [6, 10, 11, 13, 22]). This clade is joined with geographically and cladistically proximate clades to form Clade 4-4. Clade 4-4 covers a geographic area of contiguous mountain ranges roughly connected by the Muddy/Virgin River drainages. Haplotypes in tip Clade 4-3 are separated from haplotypes in interior clade 4-4 by four mutations. Haplotypes in tip clade 4-2 are separated from haplotypes in clade 4-4 by a minimum of seven mutations. Clade 4-3 is made of eight haplotypes from two localities (16, McCullough Range and 17, Eldorado Mountains). Clade 5-1 is made of 12 haplotypes from eight localities (1, 2, 4, 10, 18, 19, 20, and 24) that are found outside of the Muddy/Virgin River drainages. Clade 6-2 is made of nine haplotypes from two localities (18, Goodsprings and 21, Newberry Mountains).

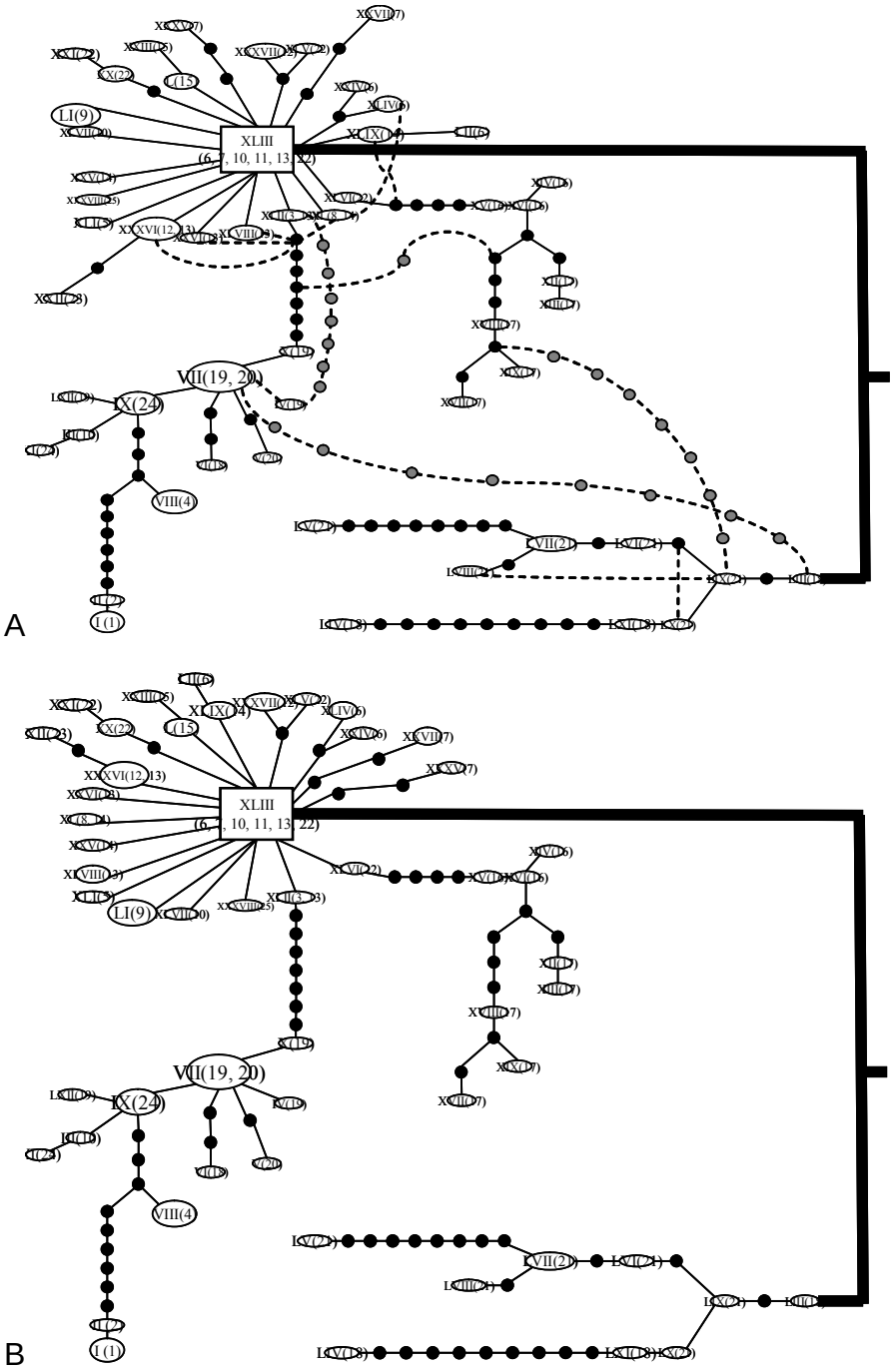

Figure 21. A) The haplotype network for combined cytochrome *b* and control region haplotypes as estimated for *Sauromalus obesus.* B) The unambiguous haplotype network for cytochrome *b* and control region haplotypes as estimated for *Sauromalus obesus* eliminating ambiguous connections after following the rules given in Templeton and Sing (1992). Each line in the network represents a single, unambiguous mutational change. Black dot indicates an interior node in the network that was not present in the sample; that is, these are inferred intermediate haplotypes between two nearest neighbor haplotypes in the network that differed by two or more mutations. Dashed lines and gray dots indicate ambiguous but equally likely connections, using the criteria in Templeton et al., (1992). Size of haplotype circles and squares is roughly proportional to haplotype frequency. Squares represent haplotypes with the greatest outgroup probability. Roman numerals represent unique haplotypes and Arabic numerals are the localities where the unique haplotypes are found. Locality numbers are listed and mapped in Figure 7.

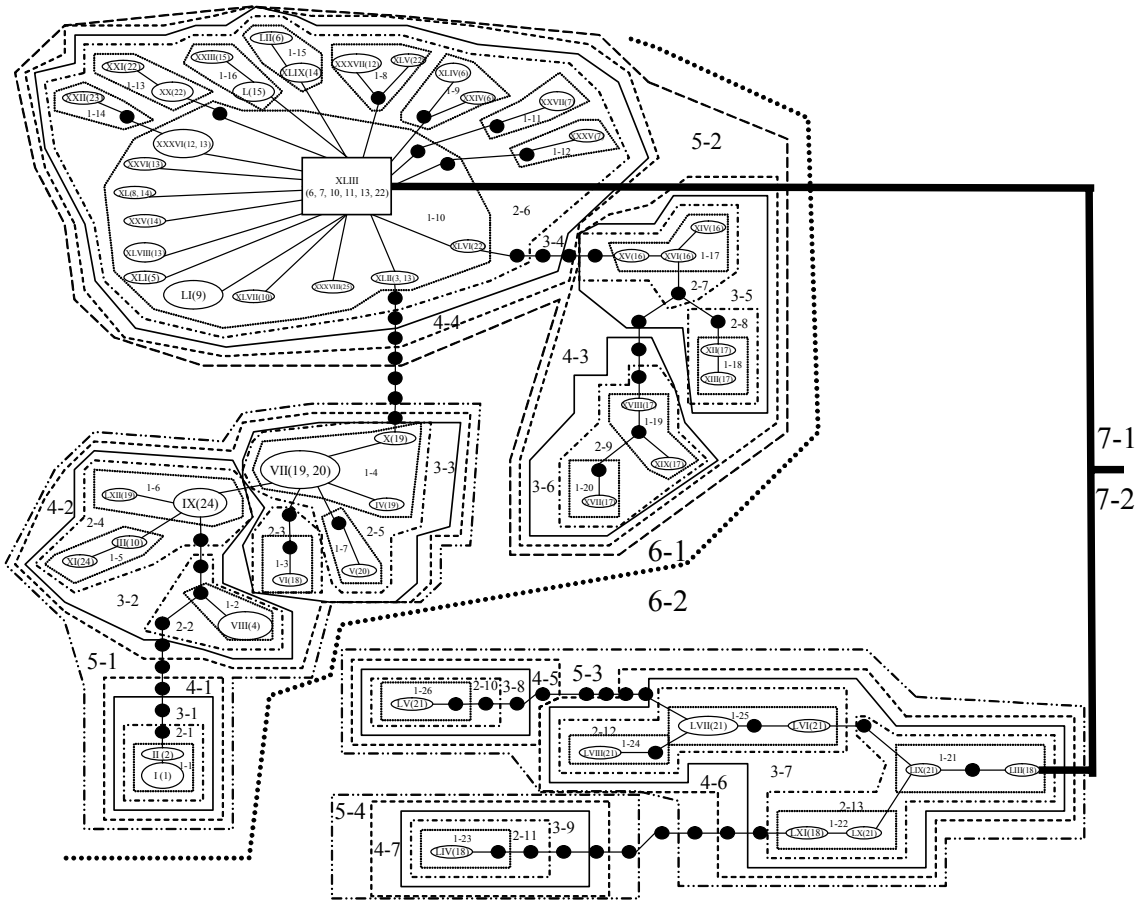

Figure 22. The haplotype network for cytochrome *b* and control region haplotypes as estimated for *Sauromalus obesus* with the nesting design of Templeton et al., (1987). Each line in the network represents a single, unambiguous mutational change. Black dots indicate an interior node in the network that was not present in the sample; that is, these are inferred intermediate haplotypes between two nearest neighbor haplotypes in the network that differed by two or more mutations. Size of haplotype circles and squares is roughly proportional to haplotype frequency. Squares represent haplotypes with the greatest outgroup probability. Roman numerals represent unique haplotypes and Arabic numerals in parentheses are the localities where the unique haplotypes are found. Arabic numerals separated by dashes indicate nested clade level and are separated by different outline patterns. Locality numbers are listed and mapped in Figure 7.

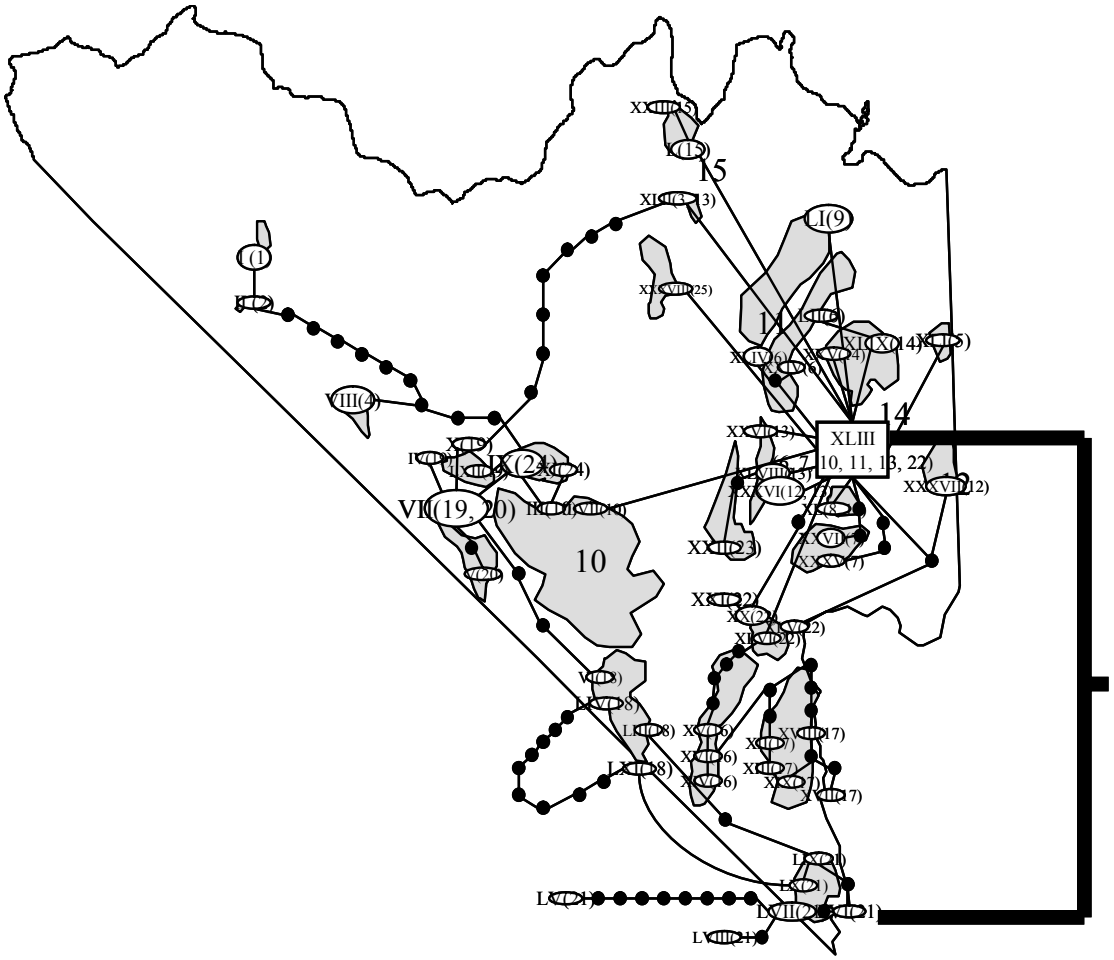

Figure 23. The haplotype network for combined cytochrome *b* and control region haplotypes as estimated for *Sauromalus obesus* overlaid on their geographic location. Each line in the network represents a single, unambiguous mutational change. Black dots indicates an interior node in the network that was not present in the sample; that is, these are inferred intermediate haplotypes between two nearest neighbor haplotypes in the network that differed by two or more mutations. Size of haplotype circles and squares is roughly proportional to haplotype frequency. Squares represent haplotypes with the greatest outgroup probability. Roman numerals represent unique haplotypes and Arabic numerals are the localities where the unique haplotypes are found. Locality numbers are listed in Figure 7.

Homogeneity testing revealed significant nonrandom association of clades and sampling locations, indicating phylogeographic structure in the data at higher clade levels (Table 10). Table 11 presents the results of the nested cladistic analysis of geographical distance for the combined fragment data set. Table 12 presents the results obtained when the inference key given in the GEODIS web site (Templeton, et al 1995) is applied to the statistical results given in Table 11 along with resulting inferences about population structure and history. Geographic distributions of clades indicate three well-supported population fragmentation events: restricted gene flow with isolation by distance, restricted gene flow/dispersal but with some long distance dispersal, and contiguous range expansion (Table 12). Restricted gene flow is found in different extents among haplotypes nested in Clades 1-1, 1-4, 1-5, 1-6, 1-10, 1-21, and 1-22, and among clades nested in Clades 2-5, 2-6, 3-7, 5-2, 5-3, and the entire cladogram. Contiguous range expansion is found among clades nested in Clades 3-2, 3-3, 3-5, 5-1 and 6-1.

Table 10. Nested contingency analysis of geographical associations for combined fragments of cytochrome *b* and control region haplotypes.

Clade	Permutational chi-square statistic	Probability
1-1	8.0	0.016
1-5	7.2	0.316
1-6	147.8	0.037
1-7	4.0	0.272
1-10	4.0	0.262
1-11	6.0	0.171
2-3	24.6	< 0.001
2-5	67.4	0.015
2-6	8.0	0.014
2-7	9.0	0.010
3-5	1.7	0.508
3-6	27.0	< 0.001
3-7	27.0	0.056
4-3	1.5	0.484
5-1	0.3	1.000
5-2	93.0	< 0.001
Entire Cladogram	99.1	< 0.001

Table 11. Results of the nested geographical analysis of *Sauromalus obesus* cytochrome *b* and control region fragments haplotypes.

Clade	Nested Clades	Position	Type of Distance	Distance	Probability <=	Probability >=
1-1	I	tip	D_c	0.00	0.00	1.00
			D_n	15.37	0.00	1.00
	II	interior	D_c	0.00	0.02	1.00
			D_n	15.37	0.98	0.02
			$D_cI - D_cT$	0.00	1.00	0.00
			$D_nI - D_nT$	0.00	0.98	0.02
1-4	IV	tip	D_c	0.00	0.21	1.00
			D_n	23.63	0.21	0.79
	VII	interior	D_c	19.29	0.17	0.91
			D_n	23.63	1.00	0.00
	X	tip	D_c	0.00	0.52	1.00
			D_n	23.63	0.52	0.48
			$D_cI - D_cT$	19.29	0.75	0.34
			$D_nI - D_nT$	0.00	0.92	0.08
1-5	XI	tip	D_c	0.00	1.00	1.00
			D_n	15.78	1.00	0.32
	III	interior	D_c	0.00	0.32	1.00
			D_n	6.31	0.00	1.00
			$D_cI - D_cT$	0.00	0.32	1.00
			$D_nI - D_nT$	-9.46	0.00	1.00
1-6	LXII	tip	D_c	0.00	1.00	1.00
			D_n	32.80	1.00	0.00
	IX	interior	D_c	0.00	0.20	1.00
			D_n	6.84	0.00	1.00
			$D_cI - D_cT$	0.00	0.20	1.00
			$D_nI - D_nT$	-25.97	0.00	1.00
1-8	XXXVII	tip	D_c	0.00	0.25	1.00
			D_n	22.85	0.25	1.00
	XLV	tip	D_c	0.00	1.00	1.00

Table 11 (continued)

Clade	Nested Clades	Position	Type of Distance	Distance	Probability <=	Probability >=
1-8	XLV	tip	D_n	58.86	1.00	0.25
1-10	XLIII	interior	D_c	40.03	0.11	0.89
			D_n	37.87	0.02	0.98
	XLVI	tip	D_c	0.00	0.76	1.00
			D_n	90.46	1.00	0.07
	XLII	tip	D_c	26.15	0.42	0.59
			D_n	68.05	0.69	0.31
	XXXVIII	tip	D_c	0.00	0.78	1.00
			D_n	73.69	0.78	0.22
	XLVII	tip	D_c	0.00	0.76	1.00
			D_n	88.42	0.88	0.12
	LI	tip	D_c	0.00	0.00	1.00
			D_n	55.02	0.38	0.62
	XLI	tip	D_c	0.00	0.79	1.00
			D_n	77.03	0.86	0.14
	XLVIII	tip	D_c	0.00	0.05	1.00
			D_n	42.78	0.16	0.84
	XXV	tip	D_c	0.00	0.78	1.00
			D_n	37.13	0.15	0.92
	XL	tip	D_c	15.49	0.23	0.79
			D_n	53.26	0.38	0.63
	XXVI	tip	D_c	0.00	0.75	1.00
			D_n	42.78	0.18	0.83
	XXXVI	tip	D_c	23.23	0.03	0.97
			D_n	62.51	0.60	0.40
			$D_cI - D_cT$	30.96	0.77	0.23
			$D_nI - D_nT$	-22.37	0.03	0.97
1-15	LII	tip	D_c	0.00	1.00	1.00
			D_n	35.13	1.00	0.25
	XLIX	interior	D_c	0.00	0.25	1.00
			D_n	11.72	0.25	1.00
			$D_cI - D_cT$	0.00	0.25	1.00
			$D_nI - D_nT$	-23.41	0.25	1.00

Table 11 (continued)

Clade	Nested Clades	Position	Type of Distance	Distance	Probability <=	Probability >=
1-21	LIII	interior	D_c	0.00	0.51	1.00
			D_n	32.17	0.00	1.00
	LIX	tip	D_c	0.00	0.49	1.00
			D_n	69.85	1.00	0.51
			$D_cI - D_cT$	0.00	0.51	0.49
			$D_nI - D_nT$	-37.68	0.00	1.00
1-22	LX	interior	D_c	0.00	0.20	1.00
			D_n	69.85	1.00	0.15
	LXI	tip	D_c	0.00	0.15	1.00
			D_n	32.17	0.00	1.00
			$D_cI - D_cT$	0.00	0.86	0.80
			$D_nI - D_nT$	37.68	1.00	0.00
2-4	1-5	tip	D_c	9.02	0.18	0.82
			D_n	14.65	0.82	0.18
	1-6	interior	D_c	11.31	0.35	0.65
			D_n	9.76	0.28	0.72
			$D_cI - D_cT$	2.30	0.45	0.55
			$D_nI - D_nT$	-4.89	0.18	0.82
2-5	1-4	interior	D_c	23.63	1.00	0.00
			D_n	23.63	0.47	0.53
	1-7	tip	D_c	0.00	0.47	1.00
			D_n	21.48	0.00	1.00
			$D_cI - D_cT$	23.63	1.00	0.00
			$D_nI - D_nT$	2.15	1.00	0.00
2-6	1-8	tip	D_c	32.93	0.21	0.80
			D_n	71.90	0.83	0.17
	1-9	tip	D_c	0.00	0.06	1.00
			D_n	18.24	0.00	1.00
	1-10	interior	D_c	57.35	0.34	0.66
			D_n	57.73	0.27	0.73
	1-11	tip	D_c	0.00	0.84	1.00

Table 11 (continued)

Clade	Nested Clades	Position	Type of Distance	Distance	Probability <=	Probability >=
			D_n	53.90	0.48	0.61
	1-12	tip	D_c	0.00	0.86	1.00
			D_n	53.90	0.46	0.64
	1-13	tip	D_c	0.00	0.01	1.00
			D_n	85.70	0.98	0.02
	1-14	tip	D_c	0.00	0.87	1.00
			D_n	61.86	0.57	0.45
	1-15	tip	D_c	17.57	0.03	0.97
			D_n	33.19	0.04	0.96
	1-16	tip	D_c	0.00	0.00	1.00
			D_n	98.66	1.00	0.00
			$D_cI - D_cT$	46.72	1.00	0.00
			$D_nI - D_nT$	-4.35	0.26	0.74
2-13	1-21	interior	D_c	44.07	0.38	0.62
			D_n	44.07	0.19	0.81
			D_c	44.07	0.19	0.81
	1-22	tip	D_n	44.07	0.19	0.81
			$D_cI - D_cT$	0.00	1.00	0.62
			$D_nI - D_nT$	0.00	0.81	0.81
3-2	2-2	tip	D_c	0.00	0.00	1.00
			D_n	46.64	0.99	0.02
	2-4	interior	D_c	11.82	0.00	1.00
			D_n	30.89	0.02	0.99
			$D_cI - D_cT$	11.82	0.83	0.17
			$D_nI - D_nT$	-15.75	0.02	0.99
3-3	2-3	tip	D_c	0.00	0.14	1.00
			D_n	76.35	1.00	0.02
	2-5	interior	D_c	23.44	0.00	1.00
			D_n	23.50	0.02	1.00
			$D_cI - D_cT$	23.44	0.66	0.34
			$D_nI - D_nT$	-52.85	0.02	1.00

Table 11 (continued)

Clade	Nested Clades	Position	Type of Distance	Distance	Probability <=	Probability >=
3-5	2-7	interior	D_c	0.00	0.02	1.00
			D_n	10.45	0.02	1.00
	2-8	tip	D_c	0.00	0.19	1.00
			D_n	20.91	1.00	0.02
			$D_cI - D_cT$	0.00	0.30	0.72
			$D_nI - D_nT$	-10.46	0.02	1.00
3-7	2-12	tip	D_c	0.00	0.00	1.00
			D_n	35.89	0.05	1.00
	2-13	interior	D_c	44.07	0.64	0.36
			D_n	56.58	0.95	0.05
			$D_cI - D_cT$	44.07	1.00	0.00
			$D_nI - D_nT$	20.69	0.95	0.05
4-2	3-2	tip	D_c	36.80	0.42	0.58
			D_n	39.61	0.64	0.36
	3-3	interior	D_c	31.63	0.11	0.89
			D_n	36.52	0.34	0.66
			$D_cI - D_cT$	-5.16	0.28	0.73
			$D_nI - D_nT$	-3.09	0.36	0.65
4-3	3-5	interior	D_c	13.94	0.19	0.93
			D_n	15.68	0.12	0.88
	3-6	tip	D_c	0.00	0.19	1.00
			D_n	15.69	1.00	0.00
			$D_cI - D_cT$	13.94	1.00	0.12
			$D_nI - D_nT$	0.00	0.12	0.88
5-1	4-1	tip	D_c	15.37	0.00	1.00
			D_n	91.57	1.00	0.00
	4-2	interior	D_c	38.23	0.00	1.00
			D_n	47.66	0.00	1.00
			$D_cI - D_cT$	22.85	0.90	0.10
			$D_nI - D_nT$	-43.91	0.00	1.00
5-2	4-3	tip	D_c	15.68	0.00	1.00

Table 11 (continued)

Clade	Nested Clades	Position	Type of Distance	Distance	Probability <=	Probability >=
5-2	4-3	tip	D_n	113.42	1.00	0.00
	4-4	interior	D_c	59.57	0.00	1.00
			D_n	60.79	0.00	1.00
			$D_cI - D_cT$	43.88	1.00	0.00
			$D_nI - D_nT$	-52.63	0.00	1.00
5-3	4-5	tip	D_c	0.00	0.16	1.00
			D_n	34.05	0.00	1.00
	4-6	interior	D_c	46.51	1.00	0.84
			D_n	45.97	0.16	0.84
			$D_cI - D_cT$	46.51	1.00	0.00
			$D_nI - D_nT$	11.91	1.00	0.00
6-1	5-1	interior	D_c	60.51	0.00	1.00
			D_n	112.03	1.00	0.00
	5-2	tip	D_c	67.35	0.00	1.00
			D_n	80.79	0.01	1.00
			$D_cI - D_cT$	-6.84	0.29	0.71
			$D_nI - D_nT$	31.24	1.00	0.00
Total	6-1	interior	D_c	90.12	0.07	0.93
			D_n	89.96	0.05	0.95
	6-2	tip	D_c	48.98	0.00	1.00
			D_n	140.39	1.00	0.00
			$D_cI - D_cT$	41.14	1.00	0.00
			$D_nI - D_nT$	-50.43	0.00	1.00

Table 12. Inference chain for cytochrome b and control region data based on results of geographic dispersion analysis given in Table 11.

Clade	Chain of Inference	Inference
Haplotypes nested in 1-1	1, 2c, 3, 5, 15, 16, 18	Geographic Sampling Scheme Inadequate to Discriminate Between Fragmentation, Range Expansion, and Isolation by Distance
Haplotypes nested in 1-4	1, 2, 11, 17, 4	Restricted Gene Flow with Isolation by Distance
Haplotypes nested in 1-5	1, 2, 11, 17, 4	Restricted Gene Flow with Isolation by Distance
Haplotypes nested in 1-6	1, 2, 11, 17, 4	Restricted Gene Flow with Isolation by Distance
Haplotypes nested in 1-8	1	Panmixia or small sample size
Haplotypes nested in 1-10	1, 2a, 3, 5, 6, 7	Restricted Gene Flow with Isolation by Distance Long Distance Dispersal
Haplotypes nested in 1-15	1	Panmixia or small sample size
Haplotypes nested in 1-21	1, 2, 11, 17, 4	Restricted Gene Flow with Isolation by Distance
Haplotypes nested in 1-22	1, 2, 11, 17, 4	Restricted Gene Flow with Isolation by Distance
One-step clades nested in 2-4	1	Panmixia or small sample size
One-step clades nested in 2-5	1, 2c, 3, 4	Restricted Gene Flow with Isolation by Distance
One-step clades nested in 2-6	1, 2a, 3, 5, 6, 7	Restricted Gene Flow/Dispersal but with some Long Distance Dispersal
One-step clades nested in 2-13	1	Panmixia or small sample size
Two-step clades nested in 3-2	1, 2, 11b, 12	Contiguous Range Expansion
Two-step clades nested in 3-3	1, 2, 11b, 12	Contiguous Range Expansion
Two-step clades nested in 3-5	1, 2, 11b, 12	Contiguous Range Expansion
Two-step clades nested in 3-7	1, 2a, 3, 5, 6, 7	Restricted Gene Flow/Dispersal but with some Long Distance Dispersal
Three-step clades nested in 4-2	1	Panmixia or small sample size
Three-step clades nested in 4-3	1, 2, 11, 17	Inconclusive outcome
Four-step clades nested in 5-1	1, 2, 11b, 12	Contiguous Range Expansion
Four-step clades nested in 5-2	1, 2c, 3, 6, 7	Restricted Gene Flow/Dispersal but with some Long Distance Dispersal
Four-step clades nested in 5-3	1, 2c, 3, 4	Restricted Gene Flow with Isolation by Distance
Five-step clades nested in 6-1	1, 2, 11b, 12	Contiguous Range Expansion
Six-step clades nested in the entire cladogram	1, 2a, 3, 5, 6, 7	Restricted Gene Flow/Dispersal but with some Long Distance Dispersal

Inference key is found in Appendix K

Appendix G

Phylogenetic analysis methods and results

Phylogenetic analyses, percent sequence divergence, and base frequencies were calculated with PAUP* 4.0b3a (Swofford, 2000). For phylogenetic analysis, each nucleotide was treated as an independent character, and character states were polarized by outgroup rooting, with the Arizona population designated as the outgroup.

It is possible to detect false phylogenetic signal in a data set simply from the stochastic nature of the sampling process (Hillis, et al., 1996). That is, if a data set were constructed by randomly assigning character sets to taxa some hierarchical structure could be detected by some phylogenetic reconstruction methods by pure chance even though there is no true hierarchical structure in the data set. Non-randomness of hierarchical structure can be assessed by examination of the shape of the distribution of tree lengths for a random sample of tree lengths (Hillis, 1991; Hillis and Huelsenbeck, 1992). Data sets with little or no hierarchical structure produce relatively symmetric tree-length frequency distribution (Fitch, 1979 and 1984), and data sets with increasing amounts of hierarchical structure become increasingly left-skewed (Hillis and Huelsenbeck, 1992). We evaluated phylogenetic signal for both genes by calculating the skewness of the distribution of tree lengths using the g1 statistic (Sokal and Rohlf, 1981) for 10,000 randomly trees generated by PAUP*.

Maximum-parsimony (MP) analysis was conducted on each data set separately with the following settings in place: ACCTRAN character-state optimization and heuristic search option; tree-bisection-reconnection (TBR) and random-addition sequence with 1000 replicates (branch swapping algorithm). Sequence data were unweighted in these analyses. Branch support for each node was evaluated using decay indices (Bremer, 1994) and non-parametric bootstrap analysis with heuristic search using 1000 for the cytochrome *b* data set and 100 replicates for the control region data set.

We also employed a maximum likelihood (ML) approach to estimate the phylogeny. We prefer the likelihood trees because maximum likelihood provides an objective basis for choosing character weights (Felsenstein, 1981), incorporates many important aspects of molecular evolution that are difficult to include using parsimony (e.g., among-site rate variation, unequal base frequencies), and has been shown to be a consistent and efficient estimator of phylogenies under a variety of simulated conditions (e.g., Huelsenbeck, 1995; Yang, 1996; Slowinski, 2001). We tested for the best fit (i.e. most appropriate) model of molecular evolution, using the MODELTEST program (version 3.04; Posada and Crandall, 1998). The MODELTEST program requires input of a simple tree (we used the default suggestion of a neighbor-joining tree, which was based on distances estimated from a Jukes-Cantor (1969) substitution model), and uses this to test 56 alternative nested models of molecular evolution for optimum fit relative to the data matrix (Appendices H, I, and J [sequences are also included] and see Huelsenbeck and Crandall, 1997; their Figure 4). The program begins by testing the data for their fit to the simplest null model (equal base frequencies, equal rates of transitions and transversions, equal rates among sites; Jukes and Cantor, 1969) versus an alternative differing by only one of these parameters [unequal base frequencies, Felsenstein, 1981; this test has 3 degrees of freedom (*df*)], and it proceeds to the next level of model

complexity after one of the original pair more "parameter simple" models has been rejected. Alternative models at the next level include another parameter (transition and transversion rates are equal, or not; 1 *df*), and successively more parameter-rich models (for example, mutation rates within transitions and within transversions are equal or not) are sequentially tested until the best fit is found (see Figure 1 of Posada and Crandall, 1998). The nested structure of the alternative models has the property, under a correct null hypothesis, of a likelihood ratio test –2 log **Ω**, which should approximate a chi-square distribution with *q* degrees of freedom (where *q* is the difference in the number of free parameters between the null model and its alternative; see Posada and Crandall, 1998). To retain the nested structure of the models, likelihood scores are estimated from the same input tree, but tree topology appears to have little influence on the likelihood estimates for a given data set in these kinds of analyses (Yang et al., 1995) and for MODELTEST in particular (da Silva et al., 2001). Once the models have been compared, a final tree is estimated using the chosen model of evolution.

The likelihood ratio tests summarized in Table 13 show that the best-fit model for the cytochrome *b* data is the TrN+G (Tamura and Nei, 1993) which was then used to estimate likelihood parameters for tree construction. The parameters input for the ML search for the cytochrome *b* data (estimated from the default NJ tree) were as follows: base frequencies = 0.2835 (A); 0.3812 (C); 0.1184 (G); 0.2169 (T); proportion of invariable sites (I) = 0; and the Gamma distribution shape parameter = 0.1179. The likelihood ratio tests summarized in Table 14 show that the best-fit model for the control region data is the TVM+I+G (Rodríguez et al., 1990), which was then used to estimate likelihood parameters for tree construction. The parameters input for the ML search for the dloop data (estimated from the default NJ tree) were as follows: base frequencies = 0.2798 (A); 0.1017 (C); 0.2494 (G); 0.3691 (T); proportion of invariable sites (I) = 0.8546; and the Gamma distribution shape parameter = 1.0384. Combining data from different genes in a likelihood framework remains a poorly explored area of phylogenetic inference (Wiens, et al. 1999). The likelihood ratio tests summarized in Table 15 show that the best-fit model for the both fragments combined data is the TIM+I+G (Rodríguez et al., 1990), which was then used to estimate likelihood parameters for tree construction. The parameters input for the ML search for both fragments combined data (estimated from the default NJ tree) were as follows: base frequencies = 0.2820 (A); 0.2255 (C); 0.1907 (G); 0.3018 (T); proportion of invariable sites (I) = 0.7883; and the Gamma distribution shape parameter = 0.6010. All ML analyses used a heuristic search strategy with TBR branch swapping, and random addition sequence for each of 10 replications; a molecular clock was not enforced. Non-parametric bootstrap analysis of 100 replicates was used to assess branch support. Samples were not assigned *a priori* as representing presumed mountain range or color pattern forms.

TCS Analysis

We then analyze the same data using a phylogeny reconstruction algorithm that is specifically designed to take advantage of the information from population genetic theory in reconstructing within-species phylogenies (Crandall, et al. 1994). It is actually more powerful when sequences differ in a few sites (Crandall and Templeton, 1996). This method has been dubbed the "TCS" method after the scientists who developed it—Alan R. Templeton, Keith A. Crandall, and Charles F. Sing (Templeton, et al., 1992). It is also known as statistical parsimony (Clement, et al., 2000). I then use the phylogeny

Table 13. MODELTEST analysis of 56 hierarchical substitution models for the cytochrome *b* fragment data; -lnL scores were estimated under various models of evolution on a neighbor-joining tree, and compared for best fit to the sequences as described by Posada and Crandall (1998). ti are nucleotide transitions and tv are nucleotide transversions.

Null model (H_0)	H_0 vs. H_1	$-\ln L_0$	$-\ln L_1$	df	*P*
Equal base frequencies	JC69[a] vs. F81[b]	1008.8172	982.1123	3	<<0.001
Equal ti/tv rates	F81[b] vs. HKY[c]	982.1123	915.5842	1	<<0.001
Equal ti rates	HKY[c] vs. TrN[d]	915.5842	900.6871	1	<<0.001
Equal tv rates	TrN[d] vs. TIM[e]	900.6871	900.6871	1	0.999
Equal rates among sites	TrN[d] vs. TrN[d] + G[f]	900.6871	886.9210	1	<<0.001
No invariable sites	TrN[d] + G[f] vs. TrN[d] + G[f] + I[g]	886.9210	885.9073	1	0.077

[a]JC69, Jukes and Cantor (1969)
[b]F81, Felsenstein (1981)
[c]HKY, Hasegawa-Kishino-Yano, (1985)
[d]TrN, Tamura and Nei (1993)
[e]TIM, (Rodríguez et al. 1990)
[f]G, shape parameter of the gamma distribution.
[g]I, proportion of invariable sites.

Table 14. MODELTEST analysis of 56 hierarchical substitution models for the control region fragment data; -lnL scores were estimated under various models of evolution on a neighbor-joining tree, and compared for best fit to the sequences as described by Posada and Crandall (1998). ti are nucleotide transitions and tv are nucleotide transversions.

Null model (H_0)	H_0 vs. H_1	$-\ln L_0$	$-\ln L_1$	df	*P*
Equal base frequencies	JC69[a] vs. F81[b]	1621.4092	1581.4857	3	<<0.001
Equal ti/tv rates	F81[b] vs. HKY[c]	1581.4857	1517.4855	1	<<0.001
Equal ti rates	HKY[c] vs. TrN[d]	1517.4855	1517.4854	1	0.987
Unequal tv rates	HKY[c] vs. K81uf[e]	1517.4855	1513.9644	1	0.008
Only two tv rates	K81uf[e] vs. TVM[f]	1513.9644	1508.4769	2	0.004
Equal rates among sites	TVM[f] vs. TVM[f] + G[g]	1508.4769	1418.5145	1	<<0.001
No invariable sites	TVM[f] + G[g] vs. TVM[f] + G[g] + I[h]	1418.5145	1390.5304	1	<<0.001

[a]JC69, Jukes and Cantor (1969)
[b]F81, Felsenstein (1981)
[c]HKY, Hasegawa-Kishino-Yano, (1985)
[d]TrN, Tamura and Nei (1993)
[e]K81uf, Kimura (1981) 3-parameters with unequal base frequencies.
[f]TVM, Transversional model (Rodríguez et al. 1990)
[g]G, shape parameter of the gamma distribution.
[h]I, proportion of invariable sites.

Table 15. MODELTEST analysis of 56 hierarchical substitution models for the combined cytochrome *b* and control region fragment data; -lnL scores were estimated under various models of evolution on a neighbor-joining tree, and compared for best fit to the sequences as described by Posada and Crandall (1998). ti are nucleotide transitions and tv are nucleotide transversions.

Null model (H_0)	H_0 vs. H_1	$-\ln L_0$	$-\ln L_1$	df	*P*
Equal base frequencies	JC69[a] vs. F81[b]	2909.8887	2892.1650	3	<<0.001
Equal ti/tv rates	F81[b] vs. HKY[c]	2892.1650	2757.2791	1	<<0.001
Equal ti rates	HKY[c] vs. TrN[d]	2757.2791	2743.9541	1	<<0.001
Unequal tv rates	TrN[d] vs. TIM[e]	2743.9541	2739.9409	1	0.005
Only two tv rates	TIM[e] vs. GTR[f]	2739.9409	2738.7913	2	0.317
Equal rates among sites	TIM[e] vs. TIM[e] + G[g]	2738.7913	2610.2310	1	<<0.001
No invariable sites	TIM[e] + G[g] vs. TIM[e] + G[g] + I[h]	2610.2310	2570.8047	1	<<0.001

[a]JC69, Jukes and Cantor (1969)
[b]F81, Felsenstein (1981)
[c]HKY, Hasegawa-Kishino-Yano, (1985)
[d]TrN, Tamura and Nei (1993)
[e]TIM, (Rodríguez et al. 1990)
[f]GTR, (Rodríguez et al. 1990)
[g]G, shape parameter of the gamma distribution.
[h]I, proportion of invariable sites.

generated from the TCS analysis with a nested analytical procedure to help separate population structure form population history (Templeton, 1998).

Intraspecific phylogenies are helpful tools in testing a variety of evolutionary and population genetic hypotheses. However, estimating genealogical relationships among genes at the population level presents a number of difficulties when using traditional methods of phylogeny reconstruction. The methods that are commonly used for reconstructing within-species phylogenies (such as parsimony, neighbor-joining, and maximum likelihood) were developed under different assumptions than what is assumed at the population level. These methods were developed under the assumption of greater evolutionary divergences such as those presumed among species, genera, and higher taxon units. Some of these assumptions are invalid at the population level (Crandall and Templeton, 1993). For example, at the species level it is assumed that haplotypes bifurcate on different trajectories through time, but this is not always necessarily the case at the population level. Genealogies at the population level often exist at or near the boundary between reticulating and diverging relationships (Baum and Shaw, 1995; Graybeal, 1995). Populations also often contain several, identical copies of a given haplotype. Each copy of the common haplotype is at risk of independent mutation (Crandall and Templeton, 1996). Therefore, it is possible that a single ancestral haplotype can give rise to multiple, descendant haplotypes (Crandall and Templeton, 1996). These events are represented as multifurcations in an estimated haplotype tree (Crandall and Templeton, 1996). It is also assumed at the species level that ancestral haplotypes are no longer in the present population. Yet at the population level not only do ancestral haplotypes persist, coalescent theory predicts that ancestral haplotypes will be the most frequent sequences sampled (Donnelly and Tavaré, 1986; Crandall and Templeton, 1993). Phylogenies reconstructed using the traditional methods are more reliable with large numbers of variable characters (Huelsenbeck and Hillis, 1993). Population studies typically lack this level of variation and our data are no exception. Furthermore, traditional methods assume recombination does not occur but this is a real possibility among sequences at the population level (Clement et al, 2000). Not incorporating the possibility of recombination can result in inaccurate phylogeny reconstruction (Clement et al, 2000), although this capability is not relevant for the chuckwalla mtDNA data set we analyzed because mtDNA does not undergo genetic recombination. The combined effects of all of these violations of assumptions at the population level can lead to a cumbersome amount of most parsimonious trees (e.g. over a million trees in this study and over one billion trees for a set of human mtDNA in Excoffier and Smouse [1994]) and over confident estimates of relationships using maximum likelihood or neighbor-joining methods (Bandelt et al, 1995). Therefore, there is reason to suspect the resolving power of traditional methods at the intraspecific level. Templeton, Crandall, and Sing (TCS) developed an alternative approach to provide accurate estimates of gene genealogies at the population level that takes into account the assumptions violated at the population level in traditional methods (Templeton et al., 1992). This method has its greatest statistical power when there are few differences and many similarities between a pair of haplotypes and it has been shown to outperform maximum parsimony when few characters are available to differentiate haplotypes (Crandall, 1994; Crandall and Templeton, 1996). TCS has been most commonly used with nucleotide data to infer population level relationships when divergences are low (humans: Templeton, 1993; salamanders: Routman et al., 1994; grasshoppers: Gerber and

Templeton, 1996; wolves: Vilá, et al. 1999). More studies should be expected with the introduction of a software package, TCS that calculates most of the algorithms that were previously done manually (Clement et al, 2000).

We use the phylogeny generated from TCS to record genealogical relationships in a manner that disentangles past and present processes (Turner, et al, 2000). While standard population level techniques such as Wright's (1969) hierarchical *F* statistics are powerful approaches for estimating gene flow, genetic drift, and population structure, they do not disentangle past events from contemporary processes. *F* statistics are used to estimate microevolutionary parameters by relating the *F* statistic to an underlying model of gene flow (Wright's "island model" or Kimura's "stepping stone" model for example). One limitation to these approaches is that the relation of F_{st} to underlying microevolutionary parameters changes with different models of population structure. To complicate things further, the data used to estimate *F* statistics often do not indicate which model of gene flow is appropriate for the population being studied. In addition, various models of gene flow are not necessarily alternatives. For example, one part of a species range may be restricted to one-dimensional (stepping stone) gene flow, while another part may be a two-dimensional (continuous, isolation by distance) model. Perhaps more pertinent to this study of chuckwallas, it is possible that the geographical genetic variation measured by F_{st} statistics may have nothing to do with current gene flow at all. For example, populations that expand into a recently colonized geographic area may show a genetic homogeneity that does not reflect their current pattern of gene flow (Larson, 1984) or two populations may have been fragmented in the past and currently have no gene flow. Yet in both scenarios shared ancestry can create F_{st} values that incorrectly imply gene flow among populations.

Geographical patterns of genetic variation are influenced by population structure, population history, and combinations of both. The TCS approach uses the inference power gained from knowing the number of haplotypes, their frequencies, and their geographical distribution such as used with standard population genetics, but also incorporates genealogical structure (Templeton et al., 1992, 1995; Templeton, 1998). This analysis uses the phylogeny estimated by statistical parsimony along with information on the geographical distributions of the various haplotypes. Geographical associations with clades in the phylogeny are statistically tested. Contingency tests are performed on each nested clade in the phylogeny against the null hypothesis of no association of clades with geographical location.

The same individuals were analyzed as explained above with the following exceptions. In the phylogenetic analysis, specimens that were identical for the regions on the genes sequences were represented as one unique haplotype. Thus, the final data set included only unique haplotypes from each of the localities sampled. Sequences in the TCS analysis are not collapsed a priori into haplotypes as frequency data is incorporated into the output (Clement et al., 2000). Therefore, all 105 individual sequences are entered into the analysis.

Probabilities of parsimony for mutational steps, the pairwise absolute distance matrix, a test listing of connections made and missing intermediates made and missing intermediates generated, and a graph description of the cladogram estimation were calculated with TCS Version 1.13 (Clement et al., 2000). Each nucleotide was treated as an independent character, and gaps were counted as events (i.e. treated as a fifth state). It was unnecessary to polarized character states by outgroup rooting. The TCS computer

program collapses sequences into haplotypes and calculates the frequencies of the haplotypes in the sample (Clement et al., 2000). The frequencies are used to estimate haplotype outgroup probabilities, which correlate with haplotype age (Donnelly and Tavaré, 1986; Castelloe and Templeton, 1994; Clement et al., 2000).

A detailed mathematical description of the algorithms used in TCS cladogram estimation can be found in Templeton et al. (1992), what follows is a conceptual summary. The algorithm not only estimates the unrooted haplotype tree but also simultaneously provides a 95% plausible set for all haplotype linkages in the unrooted tree by using a finite-site model of DNA evolution (Templeton et al., 1995). The probability of parsimony (as defined in Templeton et al. [1992], equations 6, 7, and 8) is calculated for DNA pairwise differences until the probability exceeds 0.95. The number of mutational differences associated with the probability just before the 95% cutoff is then the maximum number of mutational steps between pairs of sequences justified by the "parsimony" criterion. After all these calculations are performed, the computer program generates a graph output. In this graph, haplotypes are drawn in size proportional to their frequency. Haplotypes in a square have the greatest outgroup weight. Haplotypes and missing haplotypes are connected by branches (lines) which represent one mutational change. Some haplotypes form a closed loop connected by ambiguous linkages. Obviously, true loops cannot be created by the evolutionary process, so one or more of these plausible linkages must not have occurred (Templeton and Sing, 1993). However, it is not known which linkages did occur and which did not because all are likely (Templeton and Sing, 1993). These ambiguities are treated in the nesting design according to the rules in Templeton and Sing (1993).

Nesting design is based on the evolutionary relationships of haplotypes estimated by the TCS cladogram. The nesting rules follow Templeton et al., (1987) and Templeton and Sing (1993). Haplotypes that are separated by a single mutation are grouped together into "one step clades" proceeding from the tips to the interior of the network, then, one-step clades separated by a single mutation are grouped together into "two-step clades", etc., until the next level of nesting encompasses the entire tree (Templeton et al., 1995). Once the nested design is determined, the simplest test for geographical association treats each sample location as a categorical variable (Templeton et al., 1995). An exact permutational contingency analysis of categorical variation is then performed for any clade at each nesting level (clade types within a nested category vs. geographical location) (Templeton et al., 1995; Posada et al., 2000). A chi-square statistic is calculated from the contingency tables in which rows are genetic clades and columns are geographical locations. Another analysis is performed using geographical distance. Using the geographic locality of each population, two statistics are calculated: D_c and D_n. Clade distances (D_c), measure how geographically widespread are the individuals that bear haplotypes contained within a particular clade (Templeton et al., 1992). Clade distances are estimated by calculating the average distance that individuals bearing haplotypes grouped in a particular clade deviate from the geographic center of that clade (Templeton et al., 1995). Nested clade differences (D_n) measure how a clade is geographically distributed relative to other clades in the same higher-level nesting category. Nested clade distances are determined by calculating the average distance of observations falling within a particular haplotype class from the geographic center of the entire nesting clade. The distributions of these two distance measures under the null hypothesis of no geographical association within the nesting clade are determined by recalculating both

distances after each random permutation of clades against sampling location (Templeton et al., 1995). One thousand random permutations are performed to make statistical inference at the 5% level of significance (Edington, 1986). This allows for the testing of significantly large and small D_c and D_n distances for each clade within a nested group of clades with respect to the null hypothesis of no geographical associations within the nested clade (Templeton et al., 1995).

The joint analysis of D_c and D_n allows discrimination between short- vs. long-distance movement (either individual dispersal or population movements) (Templeton et al., 1995). Movement has been restricted to some extent if the null hypothesis has been rejected for one or both distances (Templeton et al., 1995). Long distance dispersal is inferred when D_n is greater than D_c. Short distance dispersal is implied by the two distance measures being relatively similar. These inferences are made assuming the populations under study have been adequately sampled.

Restricted gene flow is detected by having average interior clade distance minus the average tip clade distance being significantly large. This measurement is estimated by generating an interior-tip statistic (I-T) within each nested category. For the calculation of these averages, each clade distance is weighted by the number of copies in that focal clade relative to the total number of copies in the nesting clade. This tip vs. interior contrast corresponds to a young vs. old contrast and, to a lesser extent, rare vs. common (Crandall and Templeton, 1993). Significance of these statistics is estimated through a Monte Carlo procedure (Posada et al., 2000). Null distributions are constructed by randomizing the contingency data table for each clade and nesting level and estimating again the test statistics for each randomized data set (Posada et al., 2000). More detailed mathematical methods are found in Templeton et al. (1995) and Templeton (1998). Analyses are conducted using the GEODIS version 2.0 computer package (Posada et al. 2000). Once significance levels for D_c and D_n are determined, inferences about biological processes that likely gave rise to patterns of clade dispersion are made using the inference key available on-line from the GEODIS (Posada et al. 2000) web site (and presented in this report in Appendix K). This key is an updated version of the key published originally in Templeton, et al 1995). These inferences are also interpreted qualitatively in light of the historical ecology of chuckwallas.

Appendix H

PAUP commands block for MODELTEST 3.04

Cytochrome *b* fragments

```
#NEXUS
BEGIN TAXA;
DIMENSIONS  NTAX=28;
TAXLABELS
AA181CYTB
A
B
C192CYTB
D
E
F
G331CYTB
H335CYTB
I479CYTB
J391CYTB
K
L
M
N
O340CYTB
P
Q271CYTB
R
S280CYTB
T267CYTB
U
V486CYTB
W
X461CYTB
Y227CYTB
GILA731CYTB
CARBACAMEX21CYTB
;
END;


BEGIN CHARACTERS;
DIMENSIONS  NCHAR=428;
FORMAT DATATYPE=DNA  MISSING=? GAP=-;

MATRIX

AA181CYTB
GGCCACAGTCATCACCAACCTACTCTCCGCCATCCCATACGTAGGGGCCACCCTAGTAGAATG
AATCTGAGGGGGGTTCTCAGTAGATAACGCCACCCTAACTCGATTCTTCACATTTCACTTCCTA
CTGCCCTTCATAATCATCGGACTAACCATGATACACCTACTCTTCCTTCACGAAACAGGATCAA
ACAACCCAACCGGACTCAACTCCAACCCAGACAAAATCCCGTTCCATCCCTACTTCTCCTACAA
AGACCTCCTAGGAGCCACCCTAATAATTATTCTACTACTCACCCTAACCCTCTTCTCACCAAAC
CTCCTAGGCGACCCAGAAAACTTTACACCCGCCAACCCACTAGTCACACCCCCCCACATCAAG
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A
GGCCACAGTCATCACCAACCTACTCTCCGCCATCCCATACGTAGGGGCCACCCTAGTAGAATG
AATCTGAGGGGGGTTCTCAGTAGACAACGCCACCCTAACTCGATTCTTCACATTTCACTTCCTA
CTGCCCTTCATAATCATCGGACTAACCATGATACACCTACTCTTCCTTCACGAAACAGGATCAA
```

ACAACCCAACCGGACTCAACTCCAACCCAGACAAAATCCCGTTCCATCCCTACTTCTCCTACAA
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CTCCTAGGCGACCCAGAAAACTTTACACCCGCCAACCCACTAGTCACACCCCCCCACATCAAG
CCAGAATGGTATTTCCTATTCGCCTACGCCATCCTACGATCCATCC
B
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AATCTGAGGGGGGTTCTCAGTAGACAACGCCACCCTAACTCGATTCTTCACATTTCACTTCCTA
CTACCCTTCATAATCATCGGACTAACCATGATACACCTACTCTTCCTTCACGAAACAGGATCAA
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C192CYTB
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AATCTGAGGAGGGTTCTCAGTAGATAACGCCACCCTAACTCGATTCTTCACATTTCACTTCCTA
CTGCCCTTCATAATCATCGGACTAACCATGATACACCTACTCTTCCTTCACGAAACAGGATCAA
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CCAGAATGGTATTTCCTATTCGCCTACGCCATCCTACGATCCATCC
D
GGCCACAGTCATCACCAACCTACTCTCCGCCATCCCATACGTAGGGGCCACCCTAGTAGAATG
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CTGCCCTTCATAATCATCGGACTAACCATGATACACCTACTCTTCCTTCACGAAACAGGATCAA
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CTCCTAGGCGACCCAGAAAACTTTACACCCGCCAACCCACTAGTCACACCCCCCCACATCAAG
CCAGAATGGTATTTCCTATTCGCCTACGCCATCCTACGATCCATCC
E
GGCCACAGTCATCACCAACCTACTCTCCGCCATCCCATACGTAGGGGCCACCCTAGTAGAATG
AATCTGAGGGGGGTTCTCAGTAGATAACGCCACCCTAACTCGATTCTTCACATTTCACTTCCTA
CTGCCCTTCATAATCATCGGACTAACCATGATACACCTACTCTTCCTTCACGAAACAGGATCAA
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F
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AATCTGAGGGGGGTTCTCAGTAGATAACGCCACCCTAACTCGATTCTTCACATTTCACTTCCTA
CTGCCCTTCATAATCATCGGACTAACCATGATACACCTACTCTTCCTTCACGAAACAGGATCAA
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CTCCTAGGCGACCCAGAAAACTTTACACCCGCCAACCCACTAGTCACACCCCCCCACATCAAG
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G331CYTB
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AATCTGAGGGGGGTTCTCAGTAGATAACGCCACCCTAACTCGATTCTTCACATTTCACTTCCTA
CTGCCCTTCATAATCATCGGACTAACCATGATACACCTACTCTTCCTTCACGAAACAGGATCAA
ACAACCCAACCGGACTCAACTCCAACCCAGACAAAATCCCATTCCATCCCTACTTCTCCTACAA
AGACCTCCTAGGAGCCACCCTAATAATTATCCTACTACTCACCCTAGCCCTCTTCTCACCAAAC
CTCCTAGGCGACCCAGAAAACTTTACACCCGCCAACCCACTAGTCACACCCCCCCACATCAAG
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H335CYTB
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CTGCCCTTCATAATCATCGGACTAACCATGATACACCTACTCTTCCTTCACGAAACAGGATCAA
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AGACCTCCTAGGAGCCACCCTAATAATTATTCTACTACTCACCCTAGCCCTCTTCTCACCAAAC
CTCCTAAGCGACCCAGAAAACTTTACACCCGCCAACCCACTAGTCACACCCCCCCACATCAAG
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I479CYTB
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CTGCCCTTCATAATCATCGGACTAACCATGATACACCTACTCTTCCTTCACGAAACAGGATCAA
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J391CYTB
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K
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Q271CYTB
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S280CYTB
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T267CYTB
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AATCTGAGGGGGGTTCTCAGTAGATAGCGCCACCCTGACTCGATTCTTCACATTCCACTTCCTA
CTACCCTTCATAATCATCGGACTAACCATGATACACCTACTCTTCCTTCACGAAACAGGATCAA
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U
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V486CYTB
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CTGCCCTTCATAATCATCGGACTAACCATGATACACCTACTCCTCCTTCACGAAACAGGATCAA
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AGACCTCCTAGGAGCCACCCTAATAATTATTCTACTACTCACCCTAACCCTCTTCTCACCAAAC

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W
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X461CYTB
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Y227CYTB
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GATCTGAGGGGGGTTCTCAGTAGATAACGCCACCCTAACTCGATTCTTCACATTTCACTTCCTA
CTGCCCTTCATAATCATCGGACTAACCATGATACACCTACTCTTCCTTCACGAAACAGGATCAA
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CCAGAATGGTATTTCCTATTCGCCTACGCCATCCTACGATCCATCC
GILA731CYTB
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CARBACAMEX21CYTB
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ACAACCCAACCGGACTCAACTCCAACCCAGACAAAATCCCATTTCATCCCTACTTCTCCTACAA
AGACCTCCTAGGGGCCACCCTAATAATCATCCTCCTACTCACCCTAACTCTCTTCTCACCAAAC
CTCCTAGGCGACCCAGAAAACTTCACACCCGCCAACCCACTAGTCACACCCCCCCACATCAAG
CCAGAATGGTACTTTCTATTCGCCTATGCCATCCTACGATCCATCC
;
END;

[! ***** MODELFIT BLOCK -- MODELTEST 3.0 *****]
[The following command will calculate a NJ tree using the JC69 model of evolution]
BEGIN PAUP;
log file= modelfit.log replace;
DSet distance=JC objective=ME base=equal rates=equal pinv=0
subst=all negbrlen=setzero;
NJ showtree=no breakties=random;
End;

[!***** BEGIN TESTING 56 MODELS OF EVOLUTION *****]

BEGIN PAUP;

```
Set criterion=like;
[!
** Model 1 of 56 * Calculating JC **]
lscores 1/ nst=1 base=equal rates=equal pinv=0
scorefile=model.scores replace;
[!
** Model 2 of 56 * Calculating JC+I **]
lscores 1/ nst=1 base=equal rates=equal pinv=est
scorefile=model.scores append;

[!
** Model 3 of 56 * Calculating JC+G **]
lscores 1/ nst=1 base=equal rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 4 of 56 * Calculating JC+I+G **]
lscores 1/ nst=1 base=equal rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 5 of 56 * Calculating F81 **]
lscores 1/ nst=1 base=est rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 6 of 56 * Calculating F81+I **]
lscores 1/ nst=1 base=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 7 of 56 * Calculating F81+G **]
lscores 1/ nst=1 base=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 8 of 56 * Calculating F81+I+G **]
lscores 1/ nst=1 base=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 9 of 56 * Calculating K80 **]
lscores 1/ nst=2 base=equal tratio=est rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 10 of 56 * Calculating K80+I **]
lscores 1/ nst=2 base=equal tratio=est rates=equal pin=est
scorefile=model.scores append;
[!
** Model 11 of 56 * Calculating K80+G **]
lscores 1/ nst=2 base=equal tratio=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 12 of 56 * Calculating K80+I+G **]
lscores 1/ nst=2 base=equal tratio=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 13 of 56 * Calculating HKY **]
lscores 1/ nst=2 base=est tratio=est rates=equal pinv=0
scorefile=model.scores append;

[!
** Model 14 of 56 * Calculating HKY+I **]
```

```
lscores 1/ nst=2 base=est tratio=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 15 of 56 * Calculating HKY+G **]
lscores 1/ nst=2 base=est tratio=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 16 of 56 * Calculating HKY+I+G **]
lscores 1/ nst=2 base=est tratio=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 17 of 56 * Calculating TrNef **] [a b c d e f]
lscores 1/ nst=6 base=equal rmat=est rclass=(a b a a e a) rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 18 of 56 * Calculating TrNef+I **]
lscores 1/ nst=6 base=equal rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 19 of 56 * Calculating TrNef+G **]
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 20 of 56 * Calculating TrNef+I+G **]
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 21 of 56 * Calculating TrN **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 22 of 56 * Calculating TrN+I **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 23 of 56 * Calculating TrN+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 24 of 56 * Calculating TrN+I+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=est
scorefile=model.scores append;

[!
** Model 25 of 56 * Calculating K3P **] [a b c d e f]
lscores 1/ nst=6 base=equal rmat=est rclass=(a b c c b a) rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 26 of 56 * Calculating K3P+I **]
lscores 1/ nst=6 base=equal rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 27 of 56 * Calculating K3P+G **]
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 28 of 56 * Calculating K3P+I+G **]
```

```
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 29 of 56 * Calculating K3Puf **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 30 of 56 * Calculating K3Puf+I **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 31 of 56 * Calculating K3Puf+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 32 of 56 * Calculating K3Puf+I+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 33 of 56 * Calculating TIMef **] [a b c d e f]
lscores 1/ nst=6 base=equal rmat=est rclass=(a b c c e a) rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 34 of 56 * Calculating TIMef+I **]
lscores 1/ nst=6 base=equal rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 35 of 56 * Calculating TIMef+G **]

lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 36 of 56 * Calculating TIMef+I+G **]
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 37 of 56 * Calculating TIM **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 38 of 56 * Calculating TIM+I **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 39 of 56 * Calculating TIM+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 40 of 56 * Calculating TIM+I+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 41 of 56 * Calculating TVMef **] [a b c d e f]
lscores 1/ nst=6 base=equal rmat=est rclass=(a b c d b e) rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 42 of 56 * Calculating TVMef+I **]
```

```
lscores 1/ nst=6 base=equal rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 43 of 56 * Calculating TVMef+G **]
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 44 of 56 * Calculating TVMef+I+G **]
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 45 of 56 * Calculating TVM **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=0

scorefile=model.scores append;
[!
** Model 46 of 56 * Calculating TVM+I **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 47 of 56 * Calculating TVM+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 48 of 56 * Calculating TVM+I+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 49 of 56 * Calculating SYM **] [a b c d e f]
lscores 1/ nst=6 base=equal rmat=est rclass= (a b c d e f) rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 50 of 56 * Calculating SYM+I **]
lscores 1/ nst=6 base=equal rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 51 of 56 * Calculating SYM+G **]
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 52 of 56 * Calculating SYM+I+G **]
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 53 of 56 * Calculating GTR **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 54 of 56 * Calculating GTR+I **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 55 of 56 * Calculating GTR+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 56 of 56 * Calculating GTR+I+G **]
```

```
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=est

scorefile=model.scores append;

LOG STOP;
END;

[lscores: tree/s
nst: number of substitution types
rmat: rate matrix
base= nucleotide frequencies
rates= rate of evolution for variable sites (same
shape= alpha parameter of the gamma distribution
pinv= proportion of invariable sites]

[!*** END OF MODELTEST BLOCK ***]
```

Appendix I

PAUP commands block for MODELTEST 3.04

Control Region Fragments

```
#NEXUS
BEGIN TAXA;
DIMENSIONS  NTAX=47;
TAXLABELS
A
B
C403
D405
E
F
G
H331
I332
J335
K334
L
M434
N
P
Q
R
S397
T354
U486
V
W
X237
Y340
Z439
AA441
AB360
AC
AD213
AE
AF
AG485
AH377
AI483
AJ391
AK395
AL
AM
AN
AP
AQ
BB295
BC267
BD
BE280
BG
GILA731
;
END;
```

BEGIN CHARACTERS;
DIMENSIONS NCHAR=582;
FORMAT DATATYPE=DNA MISSING=? GAP=-;

MATRIX
A
????????????TAAGGCAATAGTTTCTGCTGATTATTCGTGGTTGATGTAGTATTATGTACGATAAA
GCATTTAATGTCCTATGTACGGTAAGAATTATATATTAATTAAAGAATATTCATGAGGATATTC
TCTATTGTACGATTTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTATTTAAATATTCGTGGGGAAAATAGAT
TAATGCACTATATACATAATATGTATTGGGTAAAAATGTAGTTTATAATTGTAATTGATATCCG
TGGGGAAAATAGGTTAATGCACTATATACATAGTATGTCTATAGGTGAATATCATAATATGTA
CTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGTCGAGGGGTTGCTGTTAGTTTGG
TGAGGCAAAAAAGGCCGCTCAGCGGCCTTCAGAAGATAGTTTAGTTAAAA-
TGCCAGTTTTGGGGACTGGAGACGGGGG-
TTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTCTAGGTCCCAGCTTTGGTTTACAAGACCAAT
GCTTTGTTGTTAAGCTACTAGGGCGGGATTATC
B
TTGTATTATTAATAAGGCAATAGTTTCTGTTGATTATTCGTGGTTGATGTAGTGTTATGTACGA
TAAAGCATTTAATGTCCTATGTACGGTAAGAATTATATATTAATTAAAGAATATTCATGAGGA
TATTCTCTATTGTACAATTTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTATTTAAATACTCGTGGGGAAAATAGAT
TAATGCACTATATACATAATATGTATTGGGTAAAAATGTAGTTTATAATTATGATTGATATCCG
TGGGGAAAATAGGTTAATGCACTATATACATAGTATGTCTATAGGTGAATATCATAATATGTA
CTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGTCGAGGGGTTGCTGTTAGTTTGG
TGGGGCAAAAAAGGCCGCTCAGCGGCCTTCAGAAGATAGTTTAGTTAAAA-
TGCCAGTTTTGGGGACTGGAGACGGGGG-
TTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTTTAGGTCCCAGCTTTGGTTTACAAGACCAAT
GCTTTGTTGTTAAGCTACTAGGGCGGGATTATC
C403
TTGTATTATTAATAAGGCAATAGTTTCTGTTGATTATTCGTGGTTGATGTAGTGTTATGTACGA
TAAAGCATTTAATGTCCTATGTACGGTAAGAATTATATATTAATTAAAGAATATTCATGAGGA
TATTCTCTATTGTACAATTTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTGTTTAAATATTCGTGGGGAAAATAGAT
TAATGCACTATATACATAATATGTATTGGGTAAAAATGTAGTTTATAATTATGATTGATATCCG
TGGGGAAAATAGGTTAATGCACTATATACATAGTATGTCTATAGGTGAATATCATAATATGTA
CTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGTCGAGGGGTTGCTGTTAGTTTGG
TGGGGCAAAAAAGGCCGCTGAGCGGCCTTCAGAAGATAGTTTAGTTAAAA-
TGCCAGTTTTGGGGACTGGAGACGGGGG-
TTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTTTAGGTCCCAGCTTTGGTTTACAAGACCAAT
GCTTTGTTGTTAAGCTACTAGGGCGGGATTATC
D405
TTGTATTATTAATAAGGCAATAGTTTCTGTTGATTATTCGTGGTTGATGTAGTGTTATGTACGA
TAAAGCATTTAATGTCCTATGTACGGTAAGAATTATATATTAATTAAAGAATATTCATGAGGA
TATTCTCTATTGTACAATTTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTATTTAAATATTCGTGGGGAAAATAGGT
TAATGCACTATATACATAATATGTATTGGGTAAAAATGTAGTTTATAATTATGATTGATATCCG
TGGGGAAAATAGGTTAATGCACTATATACATAGTATGTCTATAGGTGAATATCATAATATGTA
CTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGTCGAGGGGTTGCTGTTAGTTTGG
TGGGGCAAAAAAGGCCGCTCAGCGGCCTTCAGAAGATAGTTTAGTTAAAA-
TGCCAGTTTTGGGGACTGGAGACGGGGG-
TTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTTTAGGTCCCAGCTTTGGTTTACAAGACCAAT
GCTTTGTTGTTAAGCTACTAGGGCGGGATTATC
E

?TGTATTATTAATAAGGCAATAGTTTCTACTGATTATTCGTGGTTGATGTAGTATTATGTACGA
TAAAGCATTTAATGTCCTATGTACGGTAAGAATTATATATTAATTAAAGAATACTCATGAGGA
TATTCTCTATTGTACGATTTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTGTTTAAATATTCGTGGGGAAAATAGGT
TAATGCACTATATACATAATATGTATTGGGTAAAAATGTAGTTTATAATTGTAATTGATATCCG
TGGGGAAAATAGGTTAATGCACTATATACATAGTATGTCTATAGGTGAATATCATAATATGTA
CTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGTCGAGGGGTTGCTGTTAGTTTGG
TGGGGCAAAAAAGGCCGCTCAGCGGCCTTCAGAAGATAGTTTAGTTAAAA-
TGCCAGTTTTGGGGACTGGAGATGGGGG-
TTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTCTAGGTCCCAGCTTTGGTTTACAAGACCAAT
GCTTTGTTGTTAAGCTACTAGGGCGGGATTATC
F
TTGTATTGTTAATAAGGCAATAGTTTCTGCTGATTATTCGTGGTTGATGTAGTATTATGTACGA
TAAAGCATTTAATGTCCTATGTACGGTAAGAATTATATATTAATTAAAGAATATTCATGAGGA
TATTCTCTATTGTACGATTTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTATTTAAATATTCGTGGGGAAAATAGGT
TAATGCACTATATACATAATATGTATTGGGTAAAAATGTAGTTTATAATTGTAATTGATATCCG
TGGGGAAAATAGGTTAATGCACTATATACATAGTATGTCTATAGGTGAATATCATAATATGTA
CTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGTCGAGGGGTTGCTGTTAGTTTGG
TGAGGCAAAAAAGGCCGCTCAGCGGCCTTCAGAAGATAGTTTAGTTAAAA-
TGCCAGTTTTGGGGACTGGAGACGGGGG-
TTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTCTAGGTCCCAGCTTTGGTTTACAAGACCAAT
GCTTTGTTGTTAAGCTACTAGGGCGGGATTATC
G
TTGTATTATTAATAAGGCAATAGTTTCTATTGATTATTCGTGGTTGATGTAGTGTTATGTACGA
TAAAGCATTTAATGTCCTATGTACGGTAAGAATTATATATTAATTAAAGAATATTCATGAGGA
TATTCTCTATTGTACAATTTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTGTTTAAATATTCGTGGGGAAAATAGGT
TAATGCACTATATACATAATATGTATTGGGTAAAAATGTAGTTTATAATTGTGATTGATATTCG
TGGGGAAAATAGGTTAATGCACTATATACATAGTATGTCTATAGGTGAATATCATAATATGTA
CTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGTCGAGGGGTTGCTGTTAGTTTGG
TGGGGCAAAAAAGGCCGCTGAGCGGCCTTCAGAAGATAGTTTAGTTAAAA-
TGCCAGTTTTGGGGACTGGAGACGGGGG-
TTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTCTAGGTCCCAGCTTTGGTTTACAAGACCAAT
GCTTTGTTGTTAAGCTACTAGGGCGGGATTATC
H331
TTGTATTATTAATAAGGCAATAGTTTCTATTGATTATTCGTGGTTGATGTAGTGTTATGTACGA
TAAAGCATTTAATGTCCTATGTACGGTAAGAATTATATATTAATTAAAGAATATTCATGAGGA
TATTCTCTATTGTACAATTTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTGTTTAAATATTCGTGGGGAAAATAGGT
TAATGCACTATATACATAATATGTATTGGGTAAAAATGTAGTTTATAATTGTAATTGATATTCG
TGGGGAAAATAGGTTAATGCACTATATACATAGTATGTCTATAGGTGAATATCATAATATGTA
CTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGTCGAGGGGTTGCTGTTAGTTTGG
TGGGGCAAAAAAGGCCGCTGAGCGGCCTTCAGAAGATAGTTTAGTTAAAA-
TGCCAGTTTTGGGGACTGGAGACGGGGG-
TTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTCTAGGTCCCAGCTTTGGTTTACAAGACCAAT
GCTTTGTTGTTAAGCTACTAGGGCGGGATTATC
I332
TTGTATTATTAATAAGGCAATAGTTTCTATTGATTATTCATGGTTGATGTAGTGTTATGTACGA
TAAAGCATTTAATGTCCTATGTACGGTAAGAATTATATATTAACTAAAGAATATTCATGAGGA
TATTCTCTATTGTACAATTTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTGTTTAAATATTCGTGGGGAAAATAAGT
TAATGCACTATATACATAATATGTATTGGGTAAAAATGTAGTTTATAATTGTAATTGATATCCG
TGGGGAAAATAGGTTAATGCACTATATACATAGTATGTCTATAGGTGAATATCATAATATGTA
CTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGTCGAGGGGTTGCTGTTAGTTTGG
TGGGGCAAAAAAGGCCGCTGAGCGGCCTTCAGAAGATAGTTTAGTTAAAA-
TGCCAGTTTTGGGGACTGGAGATGGGGG-

TTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTCTAGGTCCCAGCTTTGGTTTACAAGACCAAT
GCTTTGTTGTTAAGCTACTAGGGCGGGATTATC
J335
??GTATTATTAATAAGGCAATAGTTTCTATTGATTATTCATGGTTGATGTAGTGTTATGTACGAT
AAAGCATTTAATGTCCTATGTACGGTAAGAATTATATATTAACTAAAGAATATTCATGAGGAT
ATTCTCTATTGTACAATTTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTGTTTAAATATTCGTGGGGAAAATAAGT
CAATGCACTATATACATAATATGTATTGGGTAAAAATGTAGTTTATAATTGTAATTGATATCCG
TGGGGAAAATAGGTTAATGCACTATATACATAGTATGTCTATAGGTGAATATCATAATATGTA
CTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGTCGAGGGGTTGCTGTTAGTTTGG
TGGGGCAAAAAAGGCCGCTGAGCGGCCTTCAGAAGATAGTTTAGTTAAAA-
TGCCAGTTTTGGGGACTGGAGATGGGGG-
TTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTCTAGGTCCCAGCTTTGGTTTACAAGACCAAT
GCTTTGTTGTTAAGCTACTAGGGCGGGATTATC
K334
?TGTATTATTAATAAGGCAATAGTTTCTGTTGATTATTCATGGTTGATGTAGTGTTATGTACGA
TAAAGCATTTAATGTCCTATGTACGGTAAGAATTATATATTAATTAAAGAATATTCATGAGGA
TATTCTCTATTGTACAATTTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTGTTTAAATATTCGTGGGGAAAATAAGT
CAATGCACTATATACATAATATGTATTGGGTAAAAATGTAGTTTATAATTGTAATTGATATCCG
TGGGGAAAATAGGTTAATGCACTATATACATAGTATGTCTATAGGTGAATATCATAATATGTA
CTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGTCGAGGGGTTGCTGTTAGTTTGG
TGGGGCAAAAAAGGCCGCTGAGCGGCCTTCAGAAGATAGTTTAGTTAAAA-
TGCCAGTTTTGGGGACTGGAGATGGGGG-
TTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTCTAGGTCCCAGCTTTGGTTTACAAGACCAAT
GCTTTGTTGTTAAGCTACTAGGGCGGGATTATC
L
TTGTATTATTAATAAGGCAATAGTTTCTGTTGATTATTCGTGGTTGATGTAGTGTTATGTACGA
TAAAGCATTTAATGTCCTATGTACGGTAAGAATTATATATTAATTAAAGAATATTCATGAGGA
TATTCTCTATTGTACAATTTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTATTTAAATATTCGTGGGGAAAATAGAT
TAATGCACTATATACATAATATGTATTGGGTAAAAATGTAGTTTATAATTATGATTGATATTCG
TGGGGAAAATAGGTTAATGCACTATATACATAGTATGTCTATAGGTGAATATCATAATATGTA
CTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGTCGAGGGGTTGCTGTTAGTTTGG
TGGGGCAAAAAAGGCCGCTCAGCGGCCTTCAGAAGATAGTTTAGTTAAAA-
TGCCAGTTTTGGGGACTGGAGACGGGGG-
TTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTTTAGGTCCCAGCTTTGGTTTACAAGACCAAT
GCTTTGTTGTTAAGCTACTAGGGCGGGATTATC
M434
TTGTATTATTAATAAGGCAATAGTTTCTGTTGATTATTCGTGGTTGATGTAGTGTTATGTACGA
TAAAGCATTTAATGTCCTATGTACGGTAAGAATTATATATTAATTAAAGAATATTCATGAGGA
TATTCTCTATTGTACAATTTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTATTTAAATATTCGTGGGGAAAATCGAT
TAATGCACTATATACATAATATGTATTGGGTAAAAATGTAGTTTATAATTATAATTGATATCCG
TGGGGAAAATAGGTTAATGCACTATATACATAGTATGTCTATAGGTGAATATCATAATATGTA
CTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGTCGAGGGGTTGCTGTTAGTTTGG
TGGGGCAAAAAAGGCCGCTCAGCGGCCTTCAGAAGATAGTTTAGTTAAAA-
TGCCAGTTTTGGGGACTGGAGACGGGGG-
TTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTTTAGGTCCCAGCTTTGGTTTACAAGACCAAT
GCTTTGTTGTTAAGCTACTAGGGCGGGATTATC
N
TTGTATTATTAATAAGGCAATAGTTTCTGTTGATTATTCGTGGTTGATGTAGTGTTATGTACGA
TAAAGCATTTAATGTCCTATGTACGGTAAGAATTATATATTAATTAAAGAATATTCATGAGGA
TATTCTCTATTGTACAATTTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTATTTAAATATTCGTGGGGAAAATAGAT
TAATGCACTATATACATAATATGTATTGGGTAAAAATGTAGTTTATAATTATGATTGATATCCG
TGGGGAAAATAGGTTAATGCACTATATACATAGTATGTCTATAGGTGAATATCATAATATGTA

CTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGTCGAGGGGTTGCTGTTAGTTTGG
TGGGGCAAAAAAGGCCGCTCAGCGGCCTTCAGAAGATAGTTTAGTTAAAA-
TGCCAGTTTTGGGGACTGGAGACGGGGG-
TTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTTTAGGTCCCAGCTTTGGTTTACAAGACCAAT
GCTTTGTTGTTAAGCTACTAGGGCGGGATTACC
P
?TGTATTATTAATAAGGCAATAGTTTCTATTGATTATTCGTGATTGATGTAGTATTATGTACGA
TAAAGCATTTAATGTCCTATGTACGGTAAGAATTATATATTAATTAAAGAATATTCATGAGGA
TATTCTCTATTGTACGATTTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTGTTTAAATATTCGTGGGGAAAATAGGT
TAATGCACTATATACATAATATGTATTGGGTAAAAATGTAGTTTATAATTATAATTGATATCCG
TGGGGAAAATAGGTTAATGCACTATATACATAGTATGTCTATAGGTGAATATCATAATATGTA
CTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGTCGAGGGGTTGCTGTTAGTTTGG
TGGGGCAAAAAAGGCCGCTCAGCGGCCTTCAGAAGATAGTTTAGTTAAAA-
TGCCAGTTTTGGGGACTGGAGATGGGGG-
TTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTTTAGGTCCCAGCTTTGGTTTACAAGACCAAT
GCTTTGTTGTTAAGCTACTAGGGCGGGGTTATC
Q
TTGTATTGTTAATAAGGCAATAGTTTCTATTGATTATTCGTGGTTGATGTAGTGTTATGTACGA
TAAAGCATTTAATGTCCTATGTACGGTAAGAATTATATATTAATTAAAGAATATTCATGAGGA
AATTCTCTATTGTACGATTTTTCATTAACGTATTTTGTTCGATAATAGGTAGATTTTACTGTTTA
AATATTCGTGGGGAAAATAAGTTAATGCACTATATACATAATATGTATTGGGTAAAAATGTAG
TTTATAATTGTTATTGATATCCGTGGGGAAAATAGGTTAATGCACTATATACATAGTATGTCTA
TAGGTGAATATCATAATATGTACTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGT
CGAGGGGTTGCTGTTAGTTTGGTGGGGCAAAAAAGGCCGCTTAGCGGCCTTCAGAAGATAGTT
TAGTTAAAAATGCCAGTTTTGGGGACTGGAGATGGGGG-
TTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTTTAGGTCCCAGCTTTGGTTTACAAGACCAAT
GCTTTGTTGTTAAGCTACTAGGGCGGGATTATC
R
??GTATTATTAATAAGGCAATAGTTTCTGTTGATTATTCGTGGTTGATGTAGTGTTATGTACGAT
AAAGCATTTAATGTCCTATGTACGGTAAGAATTATATATTAATTAAAGAATATTCATGAGGAT
ATTCTCTATTGTACAATTTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTATTTAAATATTCGTGGGGAAAATAGAT
TAATGCACTATATACATAATATGTATTGGGTAAAAATGTAGTTTATAATTATGATTGATATCCG
TGGGGAAAATAGGTTAATGCACTATATACATAGTATGTCTATAGGTGAATATCATAATATGTA
CTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGTCGAGGGGTTGCTGTTAGTTTGG
TGGGGCAAAAAAGGCCGCTGAGCGGCCTTCAGAAGATAGTTTAGTTAAAA-
TGCCAGTTTTGGGGACTGGAGACGGGGG-
TTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTTTAGGTCCCAGCTTTGGTTTACAAGACCAAT
GCTTTGTTGTTAAGCTACTAGGGCGGGATTATC
S397
TTGTATTATTAATAAGGCAATAGTTTCTGTTGATTATTCGTGGTTGATGTAGTGTTATGTACGA
TAAAGCATTTAATGTCCTATGTACGGTAAGAATTATATATTAATTAAAGAATATTCATGAGGA
TATTCTCTATTGTACAATTTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTATTTAAATATTCGTGGGGAAAATAGAT
TAATGCACTATATACATAATATGTATTGGGTAAAAATGTAGTTTATAACTATGATTGATATCCG
TGGGGAAAATAGGTTAATGCACTATATACATAGTATGTCTATAGGTGAATATCATAATATGTA
CTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGTCGAGGGGTTGCTGTTAGTTTGG
TGGGGCAAAAAAGGCCGCTCAGCGGCCTTCAGAAGATAGTTTAGTTAAAA-
TGCCAGTTTTGGGGACTGGAGACGGGGG-
TTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTTTAGGTCCCAGCTTTGGTTTACAAGACCAAT
GCTTTGTTGTTAAGCTACTAGGGCGGGATTATC
T354
CTGTATTATTAATAAGGCAATAGTTTCTATTGATTATTCGTGGTTGATGTAGTATTATGTACGA
TAAAGCATTTAATGTCCTATGTACGGTAAGAATTATATATTAATTAAAGAATATTCATGAGGA
TATTCTCTACTGTACGATTTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTGTTTAAATATTCGTGGGGAAAATAGGT

TAATGCACTATATACATAATATGTATTGGGTAAAAATGTAGTTTATAATTGTAATTGATATCCG
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TAGTTAAAAATGCCAGTTTTGGGGACTGGAGATGGGGG-
TTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTTTAGGTCCCAGCTTTGGTTTACAAGACCAAT
GCTTTGTTGTTAAGCTACTAGGGCGGGATTATC
GILA731
CTGTATTATTGATAAGGCAATAATCTCTATTGGTTATTCGTGGTTGATGTAGTGTTATGTACGA
TAAAGCATTTAATGTCCTATGTACGATAAGAATTATATATTAATTAAAGAATATTCATGAGGA
AATTCTCTATTGTACGAATTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTATTTAAATATTCGTGGGGAAAATAGAT
TAATGCACTATATACATAATATGTATTGGGTAAAAATATAGTTTATAATTGTTATTGATATCCG
TGGGGAAAATAGATTAATGCACTATATACATAGTATGTCTATAGGTGAATATCATAATATGTA
CTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGTCGAGGGGTTGCCGTTAGTTTG
GTGGGGCAAAAAAGGCCGCTAAGCGGCCTTCAGAAGATAGTTTAGTTAAAA-
TGCCAGTTTTGGGGACTGGAGATGGGGGGTTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTTT
AGGTCCCAGCTTTGGTTTACAAGACCAATGCTTTGTTGTTAAGCTACTAGGGCGGGGTTATC
;
END;

[! ***** MODELFIT BLOCK -- MODELTEST 3.0 *****]
[The following command will calculate a NJ tree using the JC69 model of evolution]

```
BEGIN PAUP;
log file= modelfit.log replace;
DSet distance=JC objective=ME base=equal rates=equal pinv=0
subst=all negbrlen=setzero;
NJ showtree=no breakties=random;
End;

[!***** BEGIN TESTING 56 MODELS OF EVOLUTION ***** ]

BEGIN PAUP;
Set criterion=like;
[!
** Model 1 of 56 * Calculating JC **]
lscores 1/ nst=1 base=equal rates=equal pinv=0
scorefile=model.scores replace;
[!
** Model 2 of 56 * Calculating JC+I **]
lscores 1/ nst=1 base=equal rates=equal pinv=est
scorefile=model.scores append;

[!
** Model 3 of 56 * Calculating JC+G **]
lscores 1/ nst=1 base=equal rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 4 of 56 * Calculating JC+I+G **]
lscores 1/ nst=1 base=equal rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 5 of 56 * Calculating F81 **]
lscores 1/ nst=1 base=est rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 6 of 56 * Calculating F81+I **]
lscores 1/ nst=1 base=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 7 of 56 * Calculating F81+G **]
lscores 1/ nst=1 base=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 8 of 56 * Calculating F81+I+G **]
lscores 1/ nst=1 base=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 9 of 56 * Calculating K80 **]
lscores 1/ nst=2 base=equal tratio=est rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 10 of 56 * Calculating K80+I **]
lscores 1/ nst=2 base=equal tratio=est rates=equal pin=est
scorefile=model.scores append;
[!
** Model 11 of 56 * Calculating K80+G **]
lscores 1/ nst=2 base=equal tratio=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
```

```
** Model 12 of 56 * Calculating K80+I+G **]
lscores 1/ nst=2 base=equal tratio=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 13 of 56 * Calculating HKY **]
lscores 1/ nst=2 base=est tratio=est rates=equal pinv=0
scorefile=model.scores append;

[!
** Model 14 of 56 * Calculating HKY+I **]
lscores 1/ nst=2 base=est tratio=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 15 of 56 * Calculating HKY+G **]
lscores 1/ nst=2 base=est tratio=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 16 of 56 * Calculating HKY+I+G **]
lscores 1/ nst=2 base=est tratio=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 17 of 56 * Calculating TrNef **] [a b c d e f]
lscores 1/ nst=6 base=equal rmat=est rclass=(a b a a e a) rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 18 of 56 * Calculating TrNef+I **]
lscores 1/ nst=6 base=equal rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 19 of 56 * Calculating TrNef+G **]
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 20 of 56 * Calculating TrNef+I+G **]
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 21 of 56 * Calculating TrN **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 22 of 56 * Calculating TrN+I **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 23 of 56 * Calculating TrN+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 24 of 56 * Calculating TrN+I+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=est
scorefile=model.scores append;

[!
** Model 25 of 56 * Calculating K3P **] [a b c d e f]
lscores 1/ nst=6 base=equal rmat=est rclass=(a b c c b a) rates=equal pinv=0
scorefile=model.scores append;
```

```
[!
** Model 26 of 56 * Calculating K3P+I **]
lscores 1/ nst=6 base=equal rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 27 of 56 * Calculating K3P+G **]
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 28 of 56 * Calculating K3P+I+G **]
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 29 of 56 * Calculating K3Puf **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 30 of 56 * Calculating K3Puf+I **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 31 of 56 * Calculating K3Puf+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 32 of 56 * Calculating K3Puf+I+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 33 of 56 * Calculating TIMef **] [a b c d e f]
lscores 1/ nst=6 base=equal rmat=est rclass=(a b c c e a) rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 34 of 56 * Calculating TIMef+I **]
lscores 1/ nst=6 base=equal rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 35 of 56 * Calculating TIMef+G **]

lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 36 of 56 * Calculating TIMef+I+G **]
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 37 of 56 * Calculating TIM **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 38 of 56 * Calculating TIM+I **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 39 of 56 * Calculating TIM+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
```

```
[!
** Model 40 of 56 * Calculating TIM+I+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 41 of 56 * Calculating TVMef **] [a b c d e f]
lscores 1/ nst=6 base=equal rmat=est rclass=(a b c d b e) rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 42 of 56 * Calculating TVMef+I **]
lscores 1/ nst=6 base=equal rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 43 of 56 * Calculating TVMef+G **]
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 44 of 56 * Calculating TVMef+I+G **]
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 45 of 56 * Calculating TVM **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=0

scorefile=model.scores append;
[!
** Model 46 of 56 * Calculating TVM+I **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 47 of 56 * Calculating TVM+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 48 of 56 * Calculating TVM+I+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 49 of 56 * Calculating SYM **] [a b c d e f]
lscores 1/ nst=6 base=equal rmat=est rclass= (a b c d e f) rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 50 of 56 * Calculating SYM+I **]
lscores 1/ nst=6 base=equal rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 51 of 56 * Calculating SYM+G **]
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 52 of 56 * Calculating SYM+I+G **]
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 53 of 56 * Calculating GTR **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=0
scorefile=model.scores append;
```

```
[!
** Model 54 of 56 * Calculating GTR+I **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 55 of 56 * Calculating GTR+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 56 of 56 * Calculating GTR+I+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=est

scorefile=model.scores append;

LOG STOP;
END;

[lscores: tree/s
nst: number of substitution types
rmat: rate matrix
base= nucleotide frequencies
rates= rate of evolution for variable sites (same
shape= alpha parameter of the gamma distribution
pinv= proportion of invariable sites]

[!*** END OF MODELTEST BLOCK ***]
```

Appendix J

PAUP commands block for MODELTEST 3.04

Control Region and Cytochrome *b* Fragments

```
#NEXUS
BEGIN TAXA;
DIMENSIONS  NTAX=56;
TAXLABELS
OUTPCU731
ALKALIFLAT01
BONNIECLAIRE03
HIKO04PCU181
BAREMTN05
BEAVER06PCU192
MEADOW07PCU441
MUDDY08PCU360
MUDDY09
MUDDY10PCU213
RAINBOW11
INDIAN12
VIRGIN13
VIRGIN14
ARROW15
ARROW16PCU403
ARROW17PCU405
MORMON18
MORMON19PCU434
MTIRISH20
MUDDY21
INDIAN22PCU397
MEADOW23PCU439
ELDORADO24PCU332
ELDORADO25PCU334
MCCULLOUGH26
MCCULLOUGH27
ELDORADO28
ELDORADO29PCU331
ELDORADO30PCU335
MTIRISH31PCU479
SPECTER32PCU391
GOODSPRINGS33
GOODSPRINGS34PCU347
NB35PCU281
GOODSPRINGS36PCU343
GOODSPRINGS37
INDIAN38
SPECTER39
LASTCHANCE40
LASTCHANCE41PCU354
MCCULLOUGH42PCU340
MEADOW43PCU237
NB44PCU271
NB45
NB46PCU280
NB47PCU267
RIVER48PCU376
RIVER49PCU483
RIVER50PCU484
```

SHEEP51PCU486
SPECTER52PCU395
SPOTTED53
SPOTTED54PCU461
TIKABOO55PCU227
RIVER57PCU377;
END;

BEGIN CHARACTERS;
DIMENSIONS NCHAR=1031;
FORMAT DATATYPE=DNA MISSING=? GAP=-;

MATRIX
OUTPCU731
CTGTATTATTGATAAGGCAATAATCTCTATTGGTTATTCGTGGTTGATGTAGTGTTATGTACGA
TAAAGCATTTAATGTCCTATGTACGATAAGAATTATATATTAATTAAAGAATATTCATGAGGA
AATTCTCTATTGTACGAATTT-
CATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTATTTAAATATTCGTGGGGAAAATAGA
TTAATGCACTATATACATAATATGTATTGGGTAAAAATATAGTTTATAATTGTTATTGATATCC
GTGGGGAAAATAGATTAATGCACTATATACATAGTATGTCTATAGGTGAATATCATAATATGT
ACTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGTCGAGGGGTTGCCGTTAGTTT
GGTGGGGCAAAAAAGGCCGCTAAGCGGCCTTCAGAAGATAGTTTAGTTAAAA-
TGCCAGTTTTGGGGACTGGAGATGGGGGGTTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTTT
AGGTCCCAGCTTTGGTTTACAAGACCAATGCTTTGTTGTTAAGCTACTAGGGCGGGGTTATC??
??????????????????AGCCACAGTCATCACCAACCTACTCTCCGCCATCCCATACGTGGGAGCCAC
CCTAGTAGAATGAATCTGAGGGGGGTTCTCAGTAGATAACGCCACCCTAACTCGATTCTTCAC
ATTTCACTTCCTACTACCCTTCATAATCATCGGACTAACCATGATACACCTACTCTTCCTTCACG
AAACAGGATCAAACAACCCAACCGGACTCAACTCCAACCCAGACAAAATCCCATTTCATCCCT
ACTTCTCCTACAAAGACCTCCTAGGGGCCACCCTAATAATTATCCTGCTACTCACCCTAACTCT
CTTCTCACCAAACCTCCTAGGCGACCCAGAAAACTTCACACCCGCCAACCCGCTAGTCACACCC
CCCCACATCAAGCCAGAATGGTACTTTCTGTTCGCCTATGCCATCCTACGATCCATCC
ALKALIFLAT01
????????????TAAGGCAATAGTTTCTGCTGATTATTCGTGGTTGATGTAGTATTATGTACGATAAA
GCATTTAATGTCCTATGTACGGTAAGAATTATATATTAATTAAAGAATATTCATGAGGATATTC
TCTATTGTACGATTTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTATTTAAATATTCGTGGGGAAAATAGAT
TAATGCACTATATACATAATATGTATTGGGTAAAAATGTAGTTTATAATTGTAATTGATATCCG
TGGGGAAAATAGGTTAATGCACTATATACATAGTATGTCTATAGGTGAATATCATAATATGTA
CTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGTCGAGGGGTTGCTGTTAGTTTGG
TGAGGCAAAAAAGGCCGCTCAGCGGCCTTCAGAAGATAGTTTAGTTAAAA-
TGCCAGTTTTGGGGACTGGAGACGGGGG-
TTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTCTAGGTCCCAGCTTTGGTTTACAAGACCAAT
GCTTTGTTGTTAAGCTACTAGGGCGGGATTATC??????????????????????GGCCACAGTCATCACC
AACCTACTCTCCGCCATCCCATACGTAGGGGCCACCCTAGTAGAATGAATCTGAGGGGGGTTC
TCAGTAGACAACGCCACCCTAACTCGATTCTTCACATTTCACTTCCTACTGCCCTTCATAATCA
TCGGACTAACCATGATACACCTACTCTTCCTTCACGAAACAGGATCAAACAACCCAACCGGAC
TCAACTCCAACCCAGACAAAATCCCGTTCCATCCCTACTTCTCCTACAAAGACCTCCTAGGAGC
CACCCTAATAATTATTCTACTACTCACCCTAGCCCTCTTCTCACCAAACCTCCTAGGCGACCCA
GAAAACTTTACACCCGCCAACCCACTAGTCACACCCCCCCACATCAAGCCAGAATGGTATTTC
CTATTCGCCTACGCCATCCTACGATCCATCC
BONNIECLAIRE03
TTGTATTGTTAATAAGGCAATAGTTTCTGCTGATTATTCGTGGTTGATGTAGTATTATGTACGA
TAAAGCATTTAATGTCCTATGTACGGTAAGAATTATATATTAATTAAAGAATATTCATGAGGA
TATTCTCTATTGTACGATTTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTATTTAAATATTCGTGGGGAAAATAGGT
TAATGCACTATATACATAATATGTATTGGGTAAAAATGTAGTTTATAATTGTAATTGATATCCG
TGGGGAAAATAGGTTAATGCACTATATACATAGTATGTCTATAGGTGAATATCATAATATGTA

CTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGTCGAGGGGTTGCTGTTAGTTTGG
TGAGGCAAAAAAGGCCGCTCAGCGGCCTTCAGAAGATAGTTTAGTTAAAA-
TGCCAGTTTTGGGGACTGGAGACGGGGG-
TTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTCTAGGTCCCAGCTTTGGTTTACAAGACCAAT
GCTTTGTTGTTAAGCTACTAGGGCGGGATTATC??????????????????????GGCCACAGTCATCACC
AACCTACTCTCCGCCATCCCATACGTAGGGGCCACCCTAGTAGAATGAATCTGAGGGGGGTTC
TCAGTAGACAACGCCACCCTAACTCGATTCTTCACATTTCACTTCCTACTGCCCTTCATAATCA
TCGGACTAACCATGATACACCTACTCTTCCTTCACGAAACAGGATCAAACAACCCAACCGGAC
TCAACTCCAACCCAGACAAAATCCCGTTCCATCCCTACTTCTCCTACAAAGACCTCCTAGGAGC
CACCCTAATAATTATTCTACTACTCACCCTAGCCCTCTTCTCACCAAACCTCCTAGGCGACCCA
GAAAACTTTACACCCGCCAACCCACTAGTCACACCCCCCCCACATCAAGCCAGAATGGTATTTC
CTATTCGCCTACGCCATCCTACGATCCATCC
HIKO04PCU181
??GTATTATTAATAAGGCAATAGTTTCTGTTGATTATTCGTGGTTGATGTAGTGTTATGTACGAT
AAAGCATTTAATGTCCTATGTACGGTAAGAATTATATATTAATTAAAGAATATTCATGAGGAT
ATTCTCTATTGTACAATTTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTATTTAAATATTCGTGGGGAAAATAGAT
TAATGCACTATATACATAATATGTATTGGGTAAAAATGTAGTTTATAATTATGATTGATATCCG
TGGGGAAAATAGGTTAATGCACTATATACATAGTATGTCTATAGGTGAATATCATAATATGTA
CTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGTCGAGGGGTTGCTGTTAGTTTGG
TGGGGCAAAAAAGGCCGCTGAGCGGCCTTCAGAAGATAGTTTAGTTAAAA-
TGCCAGTTTTGGGGACTGGAGACGGGGG-
TTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTTTAGGTCCCAGCTTTGGTTTACAAGACCAAT
GCTTTGTTGTTAAGCTACTAGGGCGGGATTATC??????????????????????GGCCACAGTCATCACC
AACCTACTCTCCGCCATCCCATACGTAGGGGCCACCCTAGTAGAATGAATCTGAGGGGGGTTC
TCAGTAGATAACGCCACCCTAACTCGATTCTTCACATTTCACTTCCTACTGCCCTTCATAATCA
TCGGACTAACCATGATACACCTACTCTTCCTTCACGAAACAGGATCAAACAACCCAACCGGAC
TCAACTCCAACCCAGACAAAATCCCGTTCCATCCCTACTTCTCCTACAAAGACCTCCTAGGAGC
CACCCTAATAATTATTCTACTACTCACCCTAACCCTCTTCTCACCAAACCTCCTAGGCGACCCA
GAAAACTTTACACCCGCCAACCCACTAGTCACACCCCCCCCACATCAAGCCAGAATGGTATTTC
CTATTCGCCTACGCCATCCTACGATCCATCC
BAREMTN05
?TGTATTATTAATAAGGCAATAGTTTCTACTGATTATTCGTGGTTGATGTAGTATTATGTACGA
TAAAGCATTTAATGTCCTATGTACGGTAAGAATTATATATTAATTAAAGAATACTCATGAGGA
TATTCTCTATTGTACGATTTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTGTTTAAATATTCGTGGGGAAAATAGGT
TAATGCACTATATACATAATATGTATTGGGTAAAAATGTAGTTTATAATTGTAATTGATATCCG
TGGGGAAAATAGGTTAATGCACTATATACATAGTATGTCTATAGGTGAATATCATAATATGTA
CTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGTCGAGGGGTTGCTGTTAGTTTGG
TGGGGCAAAAAAGGCCGCTCAGCGGCCTTCAGAAGATAGTTTAGTTAAAA-
TGCCAGTTTTGGGGACTGGAGATGGGGG-
TTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTCTAGGTCCCAGCTTTGGTTTACAAGACCAAT
GCTTTGTTGTTAAGCTACTAGGGCGGGATTATC??????????????????????GGCCACAGTCATCACC
AACCTACTCTCCGCCATCCCATACGTAGGGGCCACCCTAGTAGAATGAATCTGAGGGGGGTTC
TCAGTAGACAACGCCACCCTAACTCGATTCTTCACATTTCACTTCCTACTACCCTTCATAATCA
TCGGACTAACCATGATACACCTACTCTTCCTTCACGAAACAGGATCAAACAACCCAACCGGAC
TCAACTCCAACCCAGACAAAATCCCGTTCCATCCCTACTTCTCCTACAAAGACCTCCTAGGAGC
CACCCTAATAATTATTCTACTACTCACCCTAGCCCTCTTCTCACCAAACCTCCTAGGCGACCCA
GAAAACTTTACACCCGCCAACCCACTAGTCACACCCCCCCCACATCAAGCCAGAATGGTATTTC
CTATTCGCCTACGCCATCCTACGATCCATCC
BEAVER06PCU192
TTGTATTATTAATAAGGCAATAGTTTCTGTTGATTATTCGTGGTTGATGTAGTGTTATGTACGA
TAAAGCATTTAATGTCCTATGTACGGTAAGAATTATATATTAATTAAAGAATATTCATGAGGA
TATTCTCTATTGTACAATTTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTATTTAAATATTCGTGGGGAAAATAGAT
TAATGCACTATATACATAATATGTATTGGGTAAAAATGTAGTTTATAATTATGATTGATATCCG
TGGGGAAAATAGGTTAATGCACTATATACATAGTATGTCTATAGGTGAATATCATAATATGTA

CTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGTCGAGGGGTTGCTGTTAGTTTGG
TGGGGCAAAAAAGGCCGCTCAGCGGCCTTCAGAAGATAGTTTAGTTAAAA-
TGCCAGTTTTGGGGACTGGAGACGGGGG-
TTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTTTAGGTCCCAGCTTTGGTTTACAAGACCAAT
GCTTTGTTGTTAAGCTACTAGGGCGGGATTATC??????????????????????GGCCACAGTCATCACC
AACCTACTCTCCGCCATCCCATACGTAGGGGCCACCCTAGTAGAATGAATCTGAGGAGGGTTC
TCAGTAGATAACGCCACCCTAACTCGATTCTTCACATTTCACTTCCTACTGCCCTTCATAATCA
TCGGACTAACCATGATACACCTACTCTTCCTTCACGAAACAGGATCAAACAACCCAACCGGAC
TCAACTCCAACCCAGACAAAATCCCATTCCATCCCTACTTCTCCTACAAAGACCTCCTAGGAGC
CACCCTAATAATTATTCTACTACTCACCCTAACCCTCTTCTCACCAAACCTCCTAGGCGACCCA
GAAAACTTTACACCCGCCAACCCACTAGTCACACCCCCCCCACATCAAGCCAGAATGGTATTTC
CTATTCGCCTACGCCATCCTACGATCCATCC
MEADOW07PCU441
TTGTATTATTAATAAGGCAATAGTTTCTGTTGATTATTCGTGGTTGATGTAGTGTTATGTACGA
TAAAGCATTTAATGTCCTATGTACGGTAAGAATTATATATTAATTAAAGAATATTCATGAGGA
TATTCTCTATTGTACAATTTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTATTTAAATATTCGTGGGGAAAATAGAT
TAATGCACTATATACATAATATGTATTGGGTAAAAATGTAGTTTATAATTATGATTGATATTCG
TGGGGAAAATAGGTTAATGCACTATATACATAGTATGTCTATAGGTGAATATCATAATATGTA
CTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGTCGAGGGGTTGCTGTTAGTTTAG
TGGGGCAAAAAAGGCCGCTGAGCGGCCTTCAGAAGATAGTTTAGTTAAAA-
TGCCAGTTTTGGGGACTGGAGACGGGGG-
TTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTTTAGGTCCCAGCTTTGGTTTACAAGACCAAT
GCTTTGTTGTTAAGCTACTAGGGCGGGATTATC??????????????????????GGCCACAGTCATCACC
AACCTACTCTCCGCCATCCCATACGTAGGGGCCACCCTAGTAGAATGAATCTGAGGGGGGTTC
TCAGTAGATAACGCCACCCTAACTCGATTCTTCACATTTCACTTCCTACTGCCCTTCATAATCA
TCGGACTAACCATGATACACCTACTCTTCCTTCACGAAACAGGATCAAACAACCCAACCGGAC
TCAACTCCAACCCAGACAAAATCCCATTCCATCCCTACTTCTCCTACAAAGACCTCCTAGGAGC
CACCCTAATAATTATTCTACTACTCACCCTAACCCTCTTCTCACCAAACCTCCTAGGCGACCCA
GAAAACTTTACACCCGCCAACCCACTAGTCACACCCCCCCCACATCAAGCCAGAATGGTATTTC
CTATTCGCCTACGCCATCCTACGATCCATCC
MUDDY08PCU360
TTGTATTATTAATAAGGCAATAGTTTCTGTTGATTATTCGTGGTTGATGTAGTGTTATGTACGA
TAAAACATTTAATGTCCTATGTACGGTAAGAATTATATATTAATTAAAGAATATTCATGAGGA
TATTCTCTATTGTACAATTTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTATTATTTAAATATCCGTGGGGAAAATAGAT
TAATGCACTATATACATAATATGTATTGGGTAAAAATGTAGTTTATAATTATGATTGATATCCG
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CTATTCGCCTACGCCATCCTACGATCCATCC
RIVER57PCU377
TTGTATTATTAATAAGGCAATAGTTTCTGTTGATTATTCGTGGTTGATGTAGTGTTATGTACGA
TAAAGCATTTAATGTCCTATGTACGGTAAGAATTATATATTAATTAAAGAATATTCATGAGGA
TATTCTCTATTGTACAATTTTC-
ATTTACGTATTTTGTTCGATAATAGGTAGATTTTACTATTTAAATATTCGTGGGGAAAATAGAT
TAATGCACTATATACATAATATGTATTGGGTAAAAATGTAGTTTATAATTGTGATTGATATCCG
TGGGGAAAATAGGTTAATGCACTATATACATAGTATGTCTATAGGTGAATATCATAATATGTA
CTATGCTGCATAGGGGTGACATTTATTTAAATTTGGTGTGGTCGAGGGGTTGCTGTTAGTTTGG
TGGGGCAAAAAAGGCCGCTGAGCGGCCTTCAGAAGATAGTTTAGTTAAAA-
TGCCAGTTTTGGGGACTGGAGACGGGGG-
TTGATTCCTCTCTTTTGATGCCTTAGGGGTGGTTTAGGTCCCAGCTTTGGTTTACAAGACCAAT
GCTTTGTTGTTAAGCTACTAGGGCGGGATTATC??????????????????????GGCCACAGTCATCACC
AACCTACTCTCCGCCATCCCATACGTAGGGGCCACCCTAGTAGAATGAATCTGAGGGGGGTTC
TCAGTAGATAACGCCACCCTAACTCGATTCTTCACATTTCACTTCCTACTGCCCTTCATAATCA
TCGGACTAACCATGATACACCTACTCTTCCTTCACGAAACAGGATCAAACAACCCAACCGGAC
TCAACTCCAACCCAGACAAAATCCCATTCCATCCCTACTTCTCCTACAAAGACCTCCTAGGAGC
CACCCTAATAATTATTCTACTACTCACCCTAACCCTCTTCTCACCAAACCTCCTAGGCGACCCA
GAAAACTTTACACCCGCCAACCCACTAGTCACACCCCCCCACATCAAGCCAGAATGGTATTTC
CTATTCGCCTACGCCATCCTACGATCCATCC
;
END;

[! ***** MODELFIT BLOCK -- MODELTEST 3.0 *****]
[The following command will calculate a NJ tree using the JC69 model of evolution]
BEGIN PAUP;
log file= modelfit.log replace;
DSet distance=JC objective=ME base=equal rates=equal pinv=0
subst=all negbrlen=setzero;
NJ showtree=no breakties=random;
End;

[!***** BEGIN TESTING 56 MODELS OF EVOLUTION ***** ]

BEGIN PAUP;
Set criterion=like;
```

```
[!
** Model 1 of 56 * Calculating JC **]
lscores 1/ nst=1 base=equal rates=equal pinv=0
scorefile=model.scores replace;
[!
** Model 2 of 56 * Calculating JC+I **]
lscores 1/ nst=1 base=equal rates=equal pinv=est
scorefile=model.scores append;

[!
** Model 3 of 56 * Calculating JC+G **]
lscores 1/ nst=1 base=equal rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 4 of 56 * Calculating JC+I+G **]
lscores 1/ nst=1 base=equal rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 5 of 56 * Calculating F81 **]
lscores 1/ nst=1 base=est rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 6 of 56 * Calculating F81+I **]
lscores 1/ nst=1 base=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 7 of 56 * Calculating F81+G **]
lscores 1/ nst=1 base=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 8 of 56 * Calculating F81+I+G **]
lscores 1/ nst=1 base=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 9 of 56 * Calculating K80 **]
lscores 1/ nst=2 base=equal tratio=est rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 10 of 56 * Calculating K80+I **]
lscores 1/ nst=2 base=equal tratio=est rates=equal pin=est
scorefile=model.scores append;
[!
** Model 11 of 56 * Calculating K80+G **]
lscores 1/ nst=2 base=equal tratio=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 12 of 56 * Calculating K80+I+G **]
lscores 1/ nst=2 base=equal tratio=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 13 of 56 * Calculating HKY **]
lscores 1/ nst=2 base=est tratio=est rates=equal pinv=0
scorefile=model.scores append;

[!
** Model 14 of 56 * Calculating HKY+I **]
lscores 1/ nst=2 base=est tratio=est rates=equal pinv=est
```

```
scorefile=model.scores append;
[!
** Model 15 of 56 * Calculating HKY+G **]
lscores 1/ nst=2 base=est tratio=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 16 of 56 * Calculating HKY+I+G **]
lscores 1/ nst=2 base=est tratio=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 17 of 56 * Calculating TrNef **] [a b c d e f]
lscores 1/ nst=6 base=equal rmat=est rclass=(a b a a e a) rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 18 of 56 * Calculating TrNef+I **]
lscores 1/ nst=6 base=equal rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 19 of 56 * Calculating TrNef+G **]
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 20 of 56 * Calculating TrNef+I+G **]
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 21 of 56 * Calculating TrN **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 22 of 56 * Calculating TrN+I **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 23 of 56 * Calculating TrN+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 24 of 56 * Calculating TrN+I+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=est
scorefile=model.scores append;

[!
** Model 25 of 56 * Calculating K3P **] [a b c d e f]
lscores 1/ nst=6 base=equal rmat=est rclass=(a b c c b a) rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 26 of 56 * Calculating K3P+I **]
lscores 1/ nst=6 base=equal rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 27 of 56 * Calculating K3P+G **]
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 28 of 56 * Calculating K3P+I+G **]
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=est
```

```
scorefile=model.scores append;
[!
** Model 29 of 56 * Calculating K3Puf **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 30 of 56 * Calculating K3Puf+I **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 31 of 56 * Calculating K3Puf+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 32 of 56 * Calculating K3Puf+I+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 33 of 56 * Calculating TIMef **] [a b c d e f]
lscores 1/ nst=6 base=equal rmat=est rclass=(a b c c e a) rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 34 of 56 * Calculating TIMef+I **]
lscores 1/ nst=6 base=equal rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 35 of 56 * Calculating TIMef+G **]

lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 36 of 56 * Calculating TIMef+I+G **]
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 37 of 56 * Calculating TIM **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 38 of 56 * Calculating TIM+I **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 39 of 56 * Calculating TIM+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 40 of 56 * Calculating TIM+I+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 41 of 56 * Calculating TVMef **] [a b c d e f]
lscores 1/ nst=6 base=equal rmat=est rclass=(a b c d b e) rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 42 of 56 * Calculating TVMef+I **]
lscores 1/ nst=6 base=equal rmat=est rates=equal pinv=est
```

```
scorefile=model.scores append;
[!
** Model 43 of 56 * Calculating TVMef+G **]
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 44 of 56 * Calculating TVMef+I+G **]
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 45 of 56 * Calculating TVM **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=0

scorefile=model.scores append;
[!
** Model 46 of 56 * Calculating TVM+I **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 47 of 56 * Calculating TVM+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 48 of 56 * Calculating TVM+I+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 49 of 56 * Calculating SYM **] [a b c d e f]
lscores 1/ nst=6 base=equal rmat=est rclass= (a b c d e f) rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 50 of 56 * Calculating SYM+I **]
lscores 1/ nst=6 base=equal rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 51 of 56 * Calculating SYM+G **]
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 52 of 56 * Calculating SYM+I+G **]
lscores 1/ nst=6 base=equal rmat=est rates=gamma shape=est pinv=est
scorefile=model.scores append;
[!
** Model 53 of 56 * Calculating GTR **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=0
scorefile=model.scores append;
[!
** Model 54 of 56 * Calculating GTR+I **]
lscores 1/ nst=6 base=est rmat=est rates=equal pinv=est
scorefile=model.scores append;
[!
** Model 55 of 56 * Calculating GTR+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=0
scorefile=model.scores append;
[!
** Model 56 of 56 * Calculating GTR+I+G **]
lscores 1/ nst=6 base=est rmat=est rates=gamma shape=est pinv=est
```

```
scorefile=model.scores append;

LOG STOP;
END;

[lscores: tree/s
nst: number of substitution types
rmat: rate matrix
base= nucleotide frequencies
rates= rate of evolution for variable sites (same
shape= alpha parameter of the gamma distribution
pinv= proportion of invariable sites]

[!*** END OF MODELTEST BLOCK ***]
```

Inference Key for the Biological Interpretation of the Results of the Nested Haplotype Tree Analysis of Geographical Distances (Tables 5, 8, and 11) (Templeton, 1998)

Start with haplotypes nested within a 1-step clade:

1. Are there any significant values for D_c , D_n , or I-T within the clade?
   - NO - the null hypothesis of no geographical association of haplotypes cannot be rejected(either panmixia in sexual populations, extensive dispersal in non-sexual populations, small sample size, or inadequate geographical sampling). Move on to another clade at the same or higher level.
   - YES - Go to step 2.
2. Is at least one of the following conditions satisfied?
   a. The D_c 's for some tips are significantly small and the D_c 's for the interiors are significantly large or non-significant.
   b. The D_c 's for tips are significantly small or non-significant and the D_c 's for some but *not* all of the interiors are significantly small.
   c. The I-T D_c is significantly large.
   - NO - Go to step 11.
   - YES - Go to step 3.
   - Tip/Interior Status Cannot be Determined - **Inconclusive Outcome.**
3. Are any D_n and/or I-T D_n values significantly reversed from the D_c values, and/or do one or more tip clades show significantly large D_n 's or interior clades significantly small D_n 's or I-T significantly small D_n with the corresponding D_c values being non-significant?
   - NO - Go to step 4.
   - YES - Go to step 5.
4. Do the clades (or 2 or more subsets of them) with restricted geographical distributions have ranges that are completely or mostly non-overlapping with the other clades in the nested group (particularly interiors), and does the pattern of restricted ranges represent a break or reversal from lower level trends within the nested series (applicable to higher-level clades only)?
   - NO - **Restricted Gene Flow with Isolation by Distance (Restricted Dispersal by Distance in Non-sexual species).** This inference is strengthened if the clades with restricted distributions are found in diverse locations, if the union of their ranges roughly corresponds to the range of one or more clades (usually interiors) within the same nested group (applicable only to nesting clades with many clade members or to the highest level clades regardless of number), and if the D_c values increase and become more geographically widespread with increasing clade level within a nested series (applicable to lower level clades only).
   - YES - Go to step 9.
5. Do the clades (or 2 or more subsets of them) with restricted geographical distributions have ranges that are completely or mostly non-overlapping with the other clades in the nested group (particularly interiors), and does the pattern of restricted ranges represent a break or reversal from lower level trends within the nested series (applicable to higher-level clades only)?
   - NO - Go to step 6.
   - YES - Go to step 15.

6. Do clades (or haplotypes within them) with significant reversals or significant D_n values without significant D_c values define two or more geographically concordant subsets, or are they geographically concordant with other haplotypes/clades showing similar distance patterns?
   NO - Go to step 7.
   YES - Go to step 13.
   TOO FEW CLADES (< 2) TO DETERMINE CONCORDANCE - **Insufficient Genetic Resolution to Discriminate between Range Expansion/Colinization and Restricted Dispersal/Gene Flow** - Proceed to step 7 to determine if the geographical sampling is sufficient to discriminate between short versus long distance movement.
7. Are the clades with significantly large D_n 's (or tip clades in general when D_n for I-T is significantly small) separated from the other clades by intermediate geographical areas that were sampled?
   NO - Go to step 8.
   YES - **Restricted Gene Flow/Dispersal but with some Long Distance Dispersal.**
8. Is the species absent in the non-sampled areas?
   NO - **Sampling Design Inadequate to Discriminate between Isolation by Distance (Short Distance Movements) versus Long Distance Dispersal**
   YES - **Restricted Gene Flow/Dispersal but with some Long Distance Dispersal over Intermediate Areas not Occupied by the Species.**
9. Are the different geographical clade ranges identified in step 4 separated by areas that have not been sampled?
   NO - **Past Fragmentation.** (If inferred at a high clade level, additional confirmation occurs if the clades displaying restricted by at least partially non-overlapping distributions are mutationally connected to one another by a larger than average number of steps.)
   YES - Go to step 10.
10. Is the species absent in the non-sampled areas?
    NO - **Geographical Sampling Scheme Inadequate to Discriminate Between Fragmentation and Isolation By Distance.**
    YES - **Allopatric Fragmentation.** (If inferred at a high clade level, additional confirmation occurs if the clades displaying restricted by at least partially non-overlapping distributions are mutationally connected to one another by a larger than average number of steps.)
11. Is at least one of the following conditions satisfied?
    a. The D_c value(s) for some tip clade(s) is/are significantly large.
    b. The D_c value(s) for all interior(s) is/are significantly small.
    c. The I-T D_c is significantly small.
    NO - Go to step 17
    YES - **Range Expansion**, go to step 12.
12. Are the D_n and/or I-T D_n values significantly reversed from the D_c values?
    NO - **Contiguous Range Expansion.**
    YES - Go to step 13.

13. Are the clades with significantly large D_n 's (or tip clades in general when D_n for I-T is significantly small) separated from the geographical center of the other clades by intermediate geographical areas that were sampled?
    NO - Go to step 14.
    YES - **Long Distance Colonization**.
14. Is the species absent in the non-sampled areas?
    NO - **Sampling Design Inadequate to Discriminate between Contiguous Range Expansion and Long Distance Colonization.**
    YES - **Long Distance Colonization**.
15. Are the different geographical clade ranges identified in step 5 separated by areas that have not been sampled?
    NO - **Past Fragmentation.** (If inferred at a high clade level, additional confirmation occurs if the clades displaying restricted by at least partially non-overlapping distributions are mutationally connected to one another by a larger than average number of steps.)
    YES - Go to step 16.
16. Is the species absent in the non-sampled areas?
    NO - Go to step 18.
    YES - **Allopatric Fragmentation.** (If inferred at a high clade level, additional confirmation occurs if the clades displaying restricted by at least partially non-overlapping distributions are mutationally connected to one another by a larger than average number of steps.)
17. Are the D_n values for tip or some (but not all) interior clades significantly small, or the D_n for one or more interior clades significantly large, or is the I-T D_n value significantly large.
    NO - **Inconclusive Outcome.**
    YES - Go to step 4.
18. Are the clades found in the different geographical locations separated by a branch length with a larger than average number of mutational steps.
    NO - **Geographical Sampling Scheme Inadequate to Discriminate Between Fragmentation, Range Expansion, and Isolation By Distance.**
    YES - **Geographical Sampling Scheme Inadequate to Discriminate Between Fragmentation and Isolation By Distance.**