Population Genetics of the Native Rodents of the Galápagos Islands, Ecuador

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ABSTRACT

POPULATION GENETICS OF THE NATIVE RODENTS OF THE GALÁPAGOS ISLANDS, ECUADOR

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Reports on the native rodents of the Galápagos Islands range from anecdotal accounts to population ecology studies; however, this is the first study to examine population genetics. The genetic diversity and level of population substructure was elucidated for the 4 remaining endemic species (*Nesoryzomys swarthi, N. narboroughi, N. fernandinae*, and *Aegialomys bauri*) using microsatellites and sequences of the mtDNA d-loop. Tests for linkage disequilibrium, null allele frequency, presence of recent population bottlenecks, Hardy-Weinberg proportions, and *F*- statistics were calculated using microsatellite data. Haplotype diversity, haplotype networks, neighbor-joining phylogenetic trees, *F*- statistics, and time since most recent population expansion were calculated using the mtDNA d-loop sequences. The two locations of *Nesoryzomys swarthi* on Isla Santiago (Eastern Beach and La Bomba) represent a single population

(microsatellite F_{ST} = -0.012). Moderate substructure was documented in *Nesoryzomys narboroughi* whereas none was detected between subpopulations of *N. fernandinae* (microsatellite F_{ST} = 0.071 and 0.013, respectively). *Aegialomys bauri* exhibited great differentiation between Sampling Period 1 (collected in 1997) and Sampling Period 2 (collected in 2006), possibly representing a cyclic population bottleneck related to El Niño Southern Oscillation events (microsatellite F_{ST} = 0.158, Hederick's standardized G''_{ST} = 0.241). All species showed high d-loop haplotype diversity with low nucleotide diversity. Interestingly, *N. swarthi* was the only species to exhibit significant substructure with the d-loop (Φ_{ST} = 0.165) which may be the result of female philopatry. *Aegialomys bauri* exhibited a high number of d-loop haplotypes and a time from most recent expansion of 45,568 years, indicating it is older to than islands than originally thought. *Nesoryzomys fernandinae* had a time from most recent expansion of 116,526 years, older than the youngest age estimate of the island it currently inhabits (Isla Fernandina—60,000 yrs). These results are the first to demonstrate the level of population structure of the 4 endemic Galápagos rodent species. These data could prove useful in making recommendations for possible *ex situ* breeding programs as part of a conservation initiative in the Galápagos Islands. Further, the great temporal differentiation exhibited by *Aegialomys bauri* following an El Niño Southern Oscillation event may be a harbinger of the potential genetic impacts of global climate change.

INTRODUCTION

Insular rodents can differ from mainland rodents in behavior, demography, genetics, physiology, and morphology (Pergams and Ashley 2001). Rodents respond to evolutionary forces on islands with substantial adjustments of these characteristics and do so quickly as a result of short generation time and multiple litters per year (Pergams and Ashley 2001). Genetic factors affecting island species include isolated populations, loss of genetic and allelic variability, genetic bottlenecks, inbreeding, changes in selective pressures, or a combination of these factors. In island populations, new favorable mutations may be lost unless the coefficient of selection is very high, limiting the adaptive potential of the population (Hinten et al. 2003). Highly deleterious mutations may be 'purged' from the population via selection, but more mildly deleterious mutations can accumulate in the population due to drift (Hinten et al. 2003). Island populations are expected to suffer an increase in inbreeding (relative to the mainland population) due to bottlenecks at the foundation of the population and subsequent low average population sizes (Frankham 1998).

One example of evolution through genetic drift is the small isolated populations of endemic rodents on the Galápagos Islands. The Galápagos Islands are well known for the high percentage of endemic species found within the archipelago (Tye et al. 2002). The high level of endemism and the geographic isolation of the archipelago have made

the Galápagos Islands an ideal laboratory for studying speciation and evolution. Due to stringent collection restrictions and topological features of the islands themselves, little is known about the endemic rodents of the Galápagos.

THE GALÁPAGOS ISLANDS

The Galápagos archipelago consists of 128 islands with a total land area of 7,900 km^2 (Bensted-Smith et al. 2002). The islands range in elevation from 2 m to almost 1,700 m and in area from less than 1 m^2 to 4,600 km^2 (Tye et al. 2002). Like all oceanic archipelagos, the Galápagos are of volcanic origin. The Galápagos Islands are the result of lava plumes at the Galápagos Spreading Center at the boundary of the Cocos and Carnegie Ridges (Simkin 1984). The western islands define the hot spot with an east-southeast plate movement making the eastern islands the oldest (Simkin 1984). The surface created by lava flows is very porous and as a result the islands lack permanent running streams (Simkin 1984). Surface deposits of volcanic ash can compact to form an impermeable surface and these ash deposits are quickly eroded by run off (Simkin 1984). It is believed the Galápagos Islands are 3 to 5 million years old (Simkin 1984). Two of the larger islands, Isabela and Fernandina, are less than 1 million years old (Fernandina may be 60,000 years old; Tye et al. 2002). The oldest islands, now submerged seamounts, were previously dated at 8 to 9 million years old. A recent geochemical and volcanological study from the Galápagos hot spot tracks and adjacent seamounts puts the archipelago at an older age (Werner and Hoernle 2003). Werner and Hoernle (2003) found evidence for the continuous existence of volcanic islands above the Galápagos

hotspot for at least 17 million years. The islands are 1,000 km from the coast of Ecuador and there is no geological evidence that the islands were ever connected to mainland (Fig. 1; Simkin 1984).

The Galápagos Islands have an atypical climate for a tropical archipelago. Prevailing winds are from the southeast with the strong cold southern Peru Current cooling and drying the islands. Warmer currents from the north bring the rainy season (typically January to April). The "dry" season is generally May to December (Tye et al. 2002). El Niño Southern Oscillation events bring longer periods of warm water currents, and a more intense, prolonged rainy season (Tye et al. 2002).

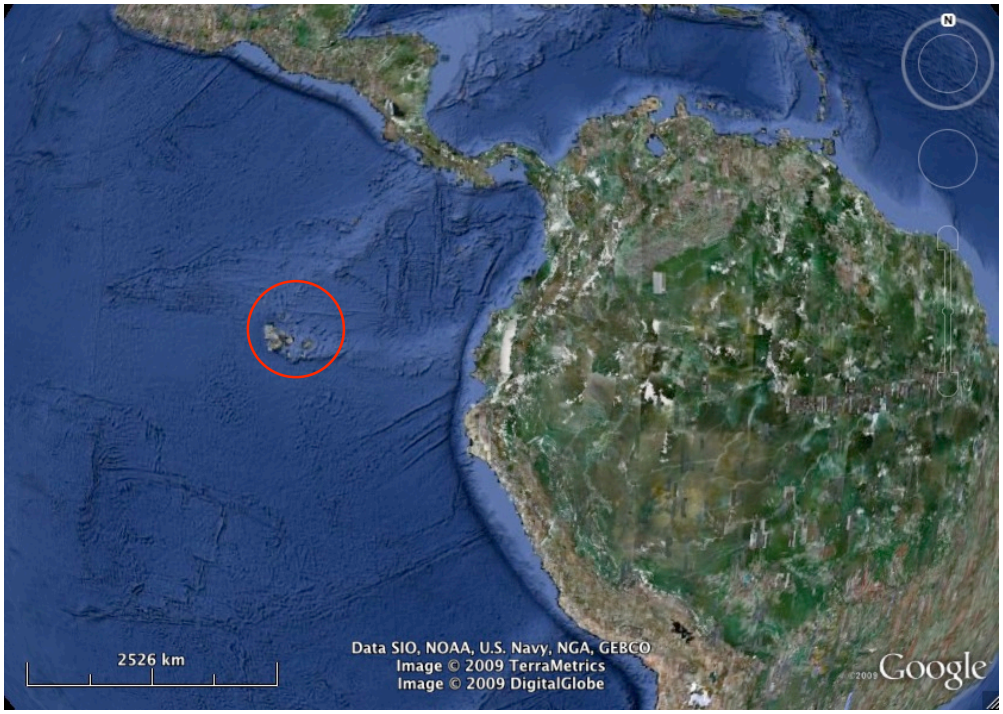

Figure 1. Location of the Galápagos Islands, relative to mainland Ecuador. (created in Google Earth)

There are 4 vegetation zones occurring throughout the archipelago: littoral, arid, transition, and humid (Tye et al. 2002). There are 560 native plant species, 32% of which are endemic (Tye et al. 2002). Sixty-seven percent of the endemic plant species are found in the lowland arid habitats with 29% and 4%, respectively, found in the humid and littoral zones (Tye et al. 2002). There are 1,822 insect species (712 endemic) and 117 species of vertebrates (84 endemic species: 40 species of reptiles—100% endemism, 58 species of birds—52% endemism, and 16 species of mammals—88% endemism).

NATIVE RODENTS

The native rodents of the Galápagos archipelago belong to the Neotropical rice rat tribe Oryzomyini (family Cricetidae, subfamily Sigmodontinae). Two genera of rodents are present on the islands: *Aegialomys* (Weksler 2006) and *Nesoryzomys* (Heller 1904). The genus *Aegialomys* includes *A. galapagoensis* (extinct, formerly from Isla San Cristobal), which has not been collected since initial capture by Darwin in 1835 and *A. bauri* (extant) of Isla Santa Fe. The genus *Nesoryzomys* comprises 5 species: *N. indefessus* from Isla Santa Cruz (extinct) and Isla Baltra, *N. darwini* from Isla Santa Cruz (extinct), *N. swarthi* from Isla Santiago (extant), and *N. narboroughi* and *N. fernandinae* from Isla Fernandina (extant; Fig. 2; Table 1; Dowler and Carroll 1996; Dowler et al. 2000). The giant rice rat, *Megaoryzomys curioi,* is known only from subfossil remains from Isla Santa Cruz and is not known to have a mainland representative (Patton and Hafner 1983). It is possible its extinction occurred prior to human settlement of the archipelago.

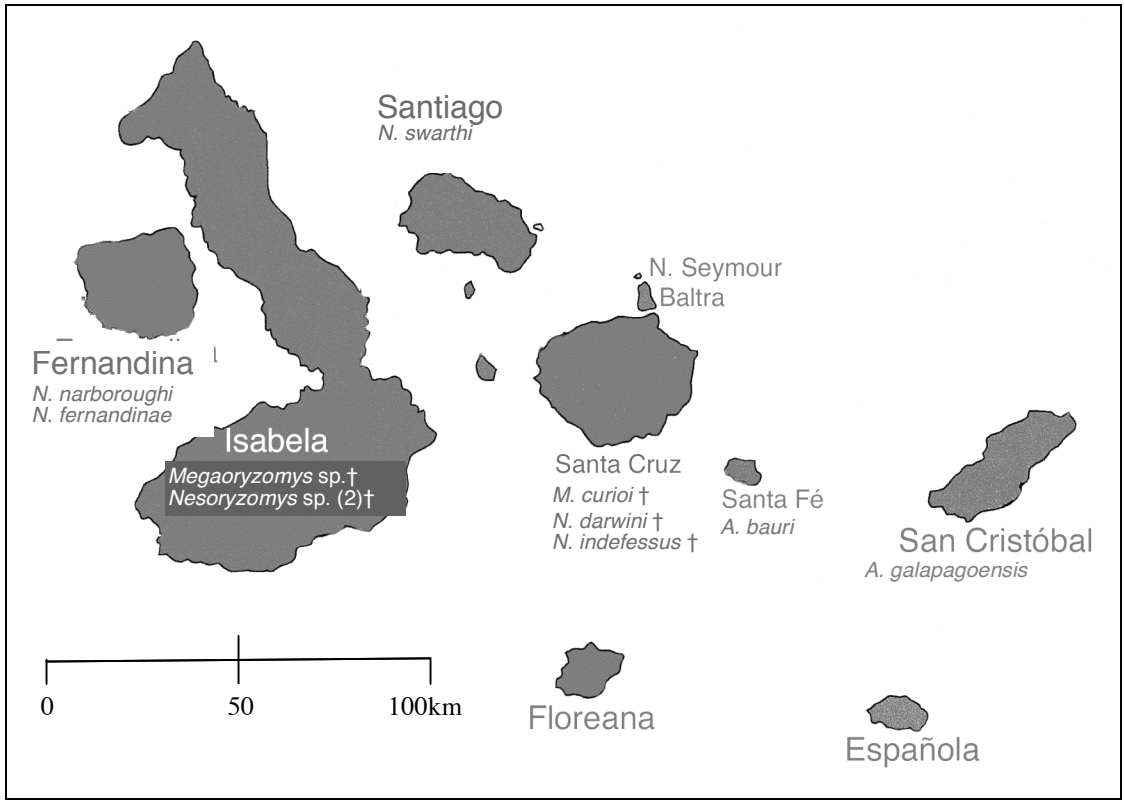

Figure 2. Map of Galápagos Islands showing locations of endemic species († indicates species now considered extinct).

Table 1. History and location of native rodents in the Galápagos Islands.

Species	Island	History	Status	Invasives present?
Megaoryzomys curioi	Santa Cruz	Never recorded alive, subfossil remains. Described by Niethammer (1964)	*Extinct*	Yes, but not relevant.
Nesoryzomys indefessus	Baltra	Originally *A. indefessus* (Thomas 1899). Re-described by Heller (1904), last collected 1929.	*Extinct*	Yes
	Santa Cruz	Recorded by Heller (1904), last collected 1934	*Extinct*	Yes
Nesoryzomys darwini	Santa Cruz	Recorded 1906, described by Osgood (1929)	*Extinct*	Yes
Nesoryzomys swarthi	Santiago	First recorded 1906, described by Orr (1938), rediscovered 1997 (Dowler et al. 2000)	**Extant**	Yes
Nesoryzomys narboroughi	Fernandina	Recorded by Heller (1904), recently recorded by Dowler et al. 2000	**Extant**	NO
Nesoryzomys fernandinae	Fernandina	Described from remains in owl pellets (Hutterer and Hirsch 1979), first live specimen recorded by Dowler and Carroll (1997)	**Extant**	NO
Aegialomys bauri	Santa Fe	Described by Allen (1892), recently recorded by Dowler et al. (2000)	**Extant**	NO
Aegialomys galapagoensis	San Cristobal	First collected by Darwin 1835, then described as *Mus galapagoensis* (Waterhouse 1839), and later re-described as *Oryzomys bauri* by Allen (1892).	*Extinct*	Yes

Systematics of the Native Rodents

The 3 genera of rodents (*Nesoryzomys, Aegialomys,* and *Megaoryzomys*) found on the Galápagos Islands are thought to result from 3 separate introductions. Both *Nesoryzomys* and *Megaoryzomys* are strongly morphologically divergent from all mainland relatives (Patton 1984). Weksler (2003) used DNA sequences for the Interphotoreceptor Retinoid Binding Protein (IRBP) sequences and 99 morphological characters to resolve the phylogenetic relationships of oryzomyines (Fig. 5). Weksler et al. (2006) included additional taxa to the previous study (Weksler 2003) and recommended generic changes for several *Oryzomys* species including the movement of *Oryzomys xanthaeolus, galapagoensis,* and *bauri* to the new genus *Aegialomys*. *Aegialomys bauri* is 'virtually identical' to *A. xanthaeolus*, an extant species on the west coast of South America (Patton 1984).

Using karyotype data from Oryzomyine rodents, Gardner and Patton (1976) determined *Nesoryzomys* to be so aberrant chromosomally as to warrant recognition as a separate genus. Smith and Patton (1999) used mtDNA cytochrome *b* sequence data to address the phylogenetic relationships of members of the subfamily Sigmodontinae. In their results, *Nesoryzomys fernandinae* and *Oryzomys* nested with other members of the tribe Oryzomyini; *Oecomys, Nectomys, Neacomys, Oligoryzomys, Microryzomys,* and *Holochilus*. Further, *Nesoryzomys* and *Nectomys* were sister taxa diverging approximately 3.5 million years ago (Smith and Patton 1999). Hanson and Bradley (2008) place *A. xanthaeolus* as sister taxa to *N. swarthi* (the only *Nesoryzomys* in their

study) used cytochrome *b*. Their findings (in conjunction with Weksler 2003; Fig. 5) suggest a single founding event by their most likely ancestor, *A. xanthaeolus*.

Description of Nesoryzomys

The genus *Nesoryzomys* is endemic to the Galápagos Islands. Based on evidence from electrophoretic and cytochrome *b* studies, the proposed origin for the genus is 3-3.5 million years before present (Patton and Hafner 1983). The genus *Nesoryzomys* is distant genetically, both karyotypically and electrophoretically, from any mainland species of rodent (Patton and Hafner 1983). The 3 extant species of *Nesoryzomys* are considered morphologically distinct (Carleton and Musser 2005).

Nesoryzomys narboroughi was first described by Heller in 1904 and is blackish in color (Orr 1938). Average adults are 77.5g and 156mm in length (Dowler and Carroll 1996). A distinguishing characteristic of *N. narboroughi* is white feet which are apparent on both adults and subadults (Dowler and Carroll 1996).

The Fernandina Galápagos mouse, *Nesoryzomys fernandinae,* was first described from owl pellet remains collected on the west side of Isla Fernandina by Hutterer and Hirsch in 1979. Though seen on the island, no complete *N. fernandinae* specimens were collected due to lack of permits. Adersen and Adersen (1987) provided an anecdotal sighting of *N. fernandinae* in 1974, but no voucher material was collected. In 1995, during an expedition to Isla Fernandina, Robert C. Dowler collected the first (known) live specimens of *N. fernandinae* (Dowler and Carroll 1996). Until this collection, this endemic rodent was thought to have been extirpated from the islands. The pelage of *N.*

fernandinae is more brown than that of *N. narboroughi* with a yellowish wash on the face, a less distinctly bicolor tail, and smaller size than *N. narboroughi* (Fig. 3). Males average 38g (weight) and 115mm (body length), whereas females average 27g and 112mm, respectively (Dowler and Carroll 1996).

The Santiago Galápagos mouse, *Nesoryzomys swarthi,* was described by Orr in 1938 based on 5 specimens collected in 1906 from Isla Santiago. This mouse is similar in size to *N. narboroughi* (~200 mm in total length and weighs approximately 90 g), but is characterized by whitish tips on the hairs of the ventral surface of the body (Fig. 3; Dowler et al. 2000). Further, the auditory bullae of *N. swarthi* are larger than *N. narboroughi* or *N. indefessus* (Orr 1938). Patton and Hafner (1983) suggested *N. swarthi* and *N. narboroughi* are actually races of *N. indefessus* and disputed their status as separate species. Though originally collected in 1906 (Orr 1938), subsequent collections yielded no specimens and this taxon was thought to have gone extinct until a small population was discovered on Isla Santiago in 1997 (Dowler et al. 2000).

Description of Aegialomys

The genus *Aegialomys* includes the extinct *A. galapagoensis* of Isla San Cristobal and *A. bauri* of Isla Santa Fe (Orr 1938). *Aegialomys galapagoensis* was described as *Mus galapagoensis* by Waterhouse in 1839, was later placed in the genus *Oryzomys* by Allen 1892, and moved to *Aegialomys* by Weksler (Carleton and Musser 2005; Weksler 2006). *Aegialomys bauri* is similar to *A. xanthaeolus* (found on mainland Ecuador) karyotypically (2n = 56; fundamental number = 58) and in electromorphic allozyme

parameters (Gardner and Patton 1976). The *Aegialomys bauri* and *galapagoensis* are now considered conspecific, though the names have been retained in scientific literature to distinguish the extant Santa Fe population from the extinct San Cristobal population. Patton and Hafner (1983) estimated an original introduction from a South American stock within the past few hundred years.

Aegialomys bauri is a small reddish brown rat, with males averaging 30% larger body size than females—males weigh 74g and are 118mm in body length, whereas females average 55g and 108mm, respectively (Fig. 3; Clark 1980). They are crepuscular/nocturnal and are often a prey item of the Galápagos short-eared owl (Clark 1984).

Figure 3. The 4 rodent species endemic to the Galápagos Islands. Clockwise from upper left: *Nesoryzomys fernandinae, N. narboroughi, Aegialomys bauri,* and *N. swarthi* (Photo courtesy of Cody W. Edwards and Robert C. Dowler).

Previous Studies Involving Native Galápagos Rodents

Previous research involving the native rodents of the Galápagos Islands includes several census surveys but few genetic studies. In 1963, Brosset reported *Aegialomys galapagoensis*, *Nesoryzomys indefessus, N. darwini,* and *N. swarthi* were extinct in the Galápagos Islands. Brosset hypothesized the extinction of the native rodents was due to competition with the invasive species, conflicts and attacks on the native rodents by *R. rattus*, and/or the introduction of diseases or parasites by invasive species (Brosset 1963). After Brosset brought 14 captive *A. bauri* from Isla Santa Fe to Isla Santa Cruz, where *R. rattus* predominated, all captive *A. bauri* had died of natural causes within one week, suggesting the introduction of disease or parasite via *R. rattus*. D. A. Clark (1984) provided a complete summary of the history of the native mammals of the Galápagos Islands, but at the time of publication little was known regarding the genus *Nesoryzomys*. Additionally, *N. swarthi* and *N. fernandinae* were both thought to be extinct at this time.

Twenty-seven Fernandina mice (*N. fernandinae*) were collected during a survey on Isla Fernandina in 1995, the first live capture of this rodent. Greatest trap success was achieved along the rim of the caldera where vegetation was the densest (Fig. 4; Dowler and Carroll 1996). Eighty-six *Nesoryzomys narboroughi* were also captured on Isla Fernandina with the greatest trap success reported in a dense stand of saltbrush (*Cryptocarpus pyriformis*) bordered by a beach. There are few areas of continuous saltbrush, which means that this preferred habitat occurs in isolated patches on the island. Both *N. fernandinae* and *N. narboroughi* were captured in the same trap lines at all sites which would indicate sympatry (Dowler and Carroll 1996). Population density of *N.*

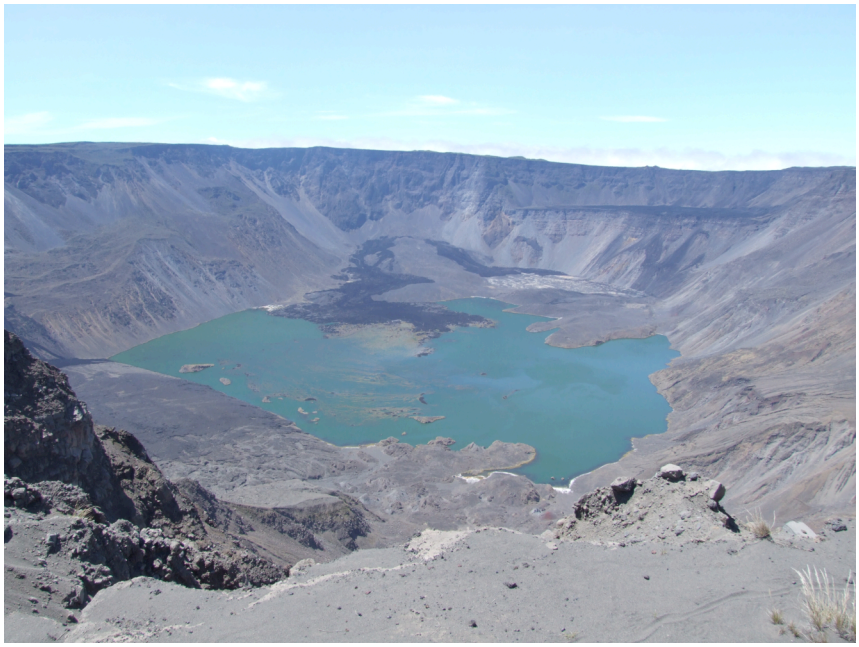

Figure 4. The Crater Rim of a volcano on Isla Fernandina. Vegetated area lies just outside the frame of view. (Photo courtesy of Cody W. Edwards)

narboroughi was estimated at 100 individuals per hectare within suitable habitat. Population density of *N. fernandinae* was estimated at 6 individuals per hectare.

An attempt to document the occurrence and distribution of the native rodents on the islands was undertaken in 1997 (Dowler et al. 2000). During this expedition, no introduced rodents were observed on Isla Santa Fe or Isla Fernandina. *Aegialomys bauri* was abundant at the collecting site on Isla Santa Fe. No evidence was found to suggest *N. indefessus* and *N. darwini* still existed on Isla Santa Cruz, only black rats (*Rattus rattus*), Norway rats (*R. norvegicus*), and house mice (*Mus musculus*) were collected. Two species of native rodents, *Nesoryzomys narboroughi* and *N. fernandinae*, were captured on Isla Fernandina. In addition, *N. swarthi* was captured on Isla Santiago,

marking the first live capture of this species since 1906. Interestingly, 2 species of introduced rodent, *R. rattus* and *M. musculus*, were also collected on Isla Santiago.

In addition to presence/absence surveys, there have been few ecological studies conducted on the islands. D. B. Clark (1980) studied the population ecology of *A. bauri* on Santa Fe using mark-recapture, morphometric measurements, reproductive condition, and distance moved over seven trapping periods totaling 3,500 trap nights during 1973-1976. Clark found the life history of *A. bauri* to be closely tied with the seasonal rains and documented a correlation between litter size and rainfall. Additionally, Clark noted over one-third of studied *A. bauri* lived to at least one year of age and two rodents lived to at least 2 years of age. Based on trap success and recapture rate, Clark estimated the population size of *A. bauri* to be 10,000 to 100,000 individuals. This is the only investigation into the ecology of *A. bauri* to date.

The most comprehensive ecological studies have focused on *N. swarthi*. Using trapping grids, mark recapture, and radio telemetry Harris and MacDonald (2007) provided the first comprehensive ecological study of *N. swarthi*. *Nesoryzomys swarthi* abundance was positively correlated with cactus density and to a lesser extent shrub density (Harris and MacDonald 2007). Breeding by *N. swarthi* spanned the wet season with a peak in pregnancy and lactation in April. Median survivorship was 199 days for males and 203 days for females, with a record of 812 days for a female. The sex ratio was skewed toward males (56:43). Male home ranges were larger than females (2.79 ha and 0.99 ha, respectively) and typically overlapped multiple female ranges suggesting a promiscuous or polygynous mating strategy. Additionally, Harris et al. (2006) used radio

tracking, live trapping, and spool-and-line tracking to quantify the abundance and habitat correlations of *N. swarthi* and *R. rattus* on Isla Santiago. *Nesoryzomys swarthi* currently is restricted to a 14 km stretch of north central coast on the island while *R. rattus* was collected at all trap sites throughout the island. *Nesoryzomys swarthi* density did not vary with local density of *R. rattus*. However, there was an increase in *N. swarthi* activity during pre-dawn and post dusk hours with increased *R. rattus* density, exhibiting an increase in *N. swarthi* activity during periods of low *R. rattus* activity.

Gregory and MacDonald (2009) presented the first description of *N. swarthi* feeding habits. The diet habits of *R. rattus* and *N. swarthi* were elucidated using spool and line to track visitation to plants and by preferred diet trials in temporary captivity during both wet and dry seasons. Foods selected by both species were highly seasonal with almost complete overlap, with the exception of *Opuntia galapageia* cactus fruit, which was not eaten by *R. rattus*, but were selected (though not preferred) by *N. swarthi*. Female *N. swarthi* exhibited a broader selected diet than males probably due to reproductive induced energy requirements. Although diet overlap was high between the native and invasive rodents, *R. rattus* tended to select fruits year-round while *N. swarthi* selected *Opuntia* foods. *Nesoryzomys swarthi* exhibited a narrow selected diet breadth in the wet season that broadened in the dry season. The 2 species may be successfully coexisting as a result of the behaviorally subordinate species (*N. swarthi*) surviving better in a secondary habitat than the dominant species (*R. rattus*). As the competitive pressure exerted by *R. rattus* declined due to population crashes in the dry season, *N. swarthi* were able to select a broader range of foods.

The phylogenetic relationship of the endemic species has been superficially investigated, though a comprehensive study is still needed. Patton and Hafner (1983) examined the cranial morphology and external morphometrics of *Nesoryzomys narboroughi, N. swarthi, N. darwini, N. indefessus,* and *A. bauri* as well as the karyotype of *N. narboroughi* and *A. bauri*. Based on data from 22 protein loci, *A. bauri* and *A. xanthaeolus* share 86.4% similarity, with morphological data suggesting the ancestral stock of *Nesoryzomys* is related to *A. xanthaeolus*. Patton and Hafner (1983) suggested *N. narboroughi* and *N. swarthi* were not morphologically distinct from *N. indefessus* aside from pelage color and did not warrant specific recognition. These authors considered *N. narboroughi* and *N. swarthi* to be moderately delineated races of *N. indefessus*, of which *indefessus* has taxonomic priority. Although *N. swarthi* is now recognized as a distinct species, *N. narboroughi* is listed as a subspecies of *N. indefessus*, and no subsequent studies have addressed the systematics of all extant taxa (Carleton and Musser 2005; Dowler et al. 2000).

Weksler (2003) sequenced the first exon of the nuclear gene encoding the Interphotoreceptor Retinoid Binding Protein (IRBP) in 44 species representing 16 genera within the tribe Oryzomyini in the Family Sigmodontinae (Fig. 5). Data from these analyses revealed 2 distinct clades. The first clade within Oryzomyini included several species of *Aegialomys* and *Oecomys*. The second clade included the remaining *Aegialomys* species and additional genera and was further divided into 2 poorly supported clades, one of which included *Nesoryzomys swarthi* and *Nesoryzomys narboroughi, Melanomys caliginosus, A. xanthaeolus* (thought to be the recent ancestor of *A. bauri*),

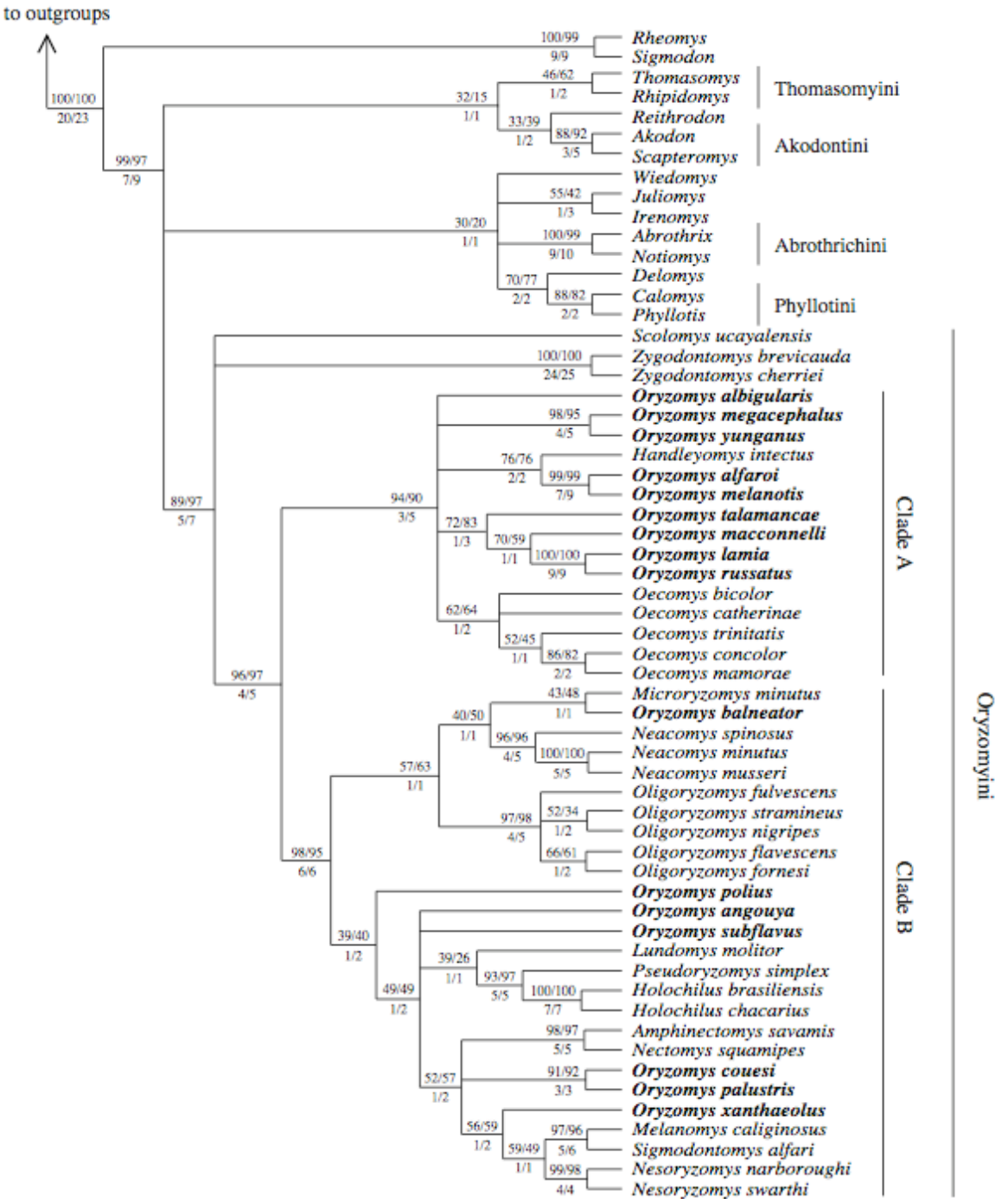

Figure 5. Strict consensus from cladistic parsimony analysis of IRBP DNA sequences of Neotropical Oryzomyine rodents (Weksler 2003, with permission).

Oryzomys palustris, O. cousei, Nectomys squamipes, and *Amphinectomys savamis*. Weksler did not include *N. fernandinae* and *A. bauri* in his study.

HUMAN IMPACTS

The Galápagos Islands are well known for their high percentage of endemic species as well as their geographic isolation. Unfortunately, the latter attribute is no longer shielding the islands from anthropogenic influences.

Human Settlements

One of the earliest documented visits to the Galápagos Islands was in 1535 A.D. by the Bishop of Panamá (Steadman et al. 1991). Although pirates and whalers visited the islands sporadically in the following centuries, the first permanent settlement was not established until 1832 (Tye et al. 2002). A noticeable acceleration in population growth began in the 1950s (Snell et al. 2002). This increase in immigration to the islands likely was caused by the economic incentives resulting from increased tourism (Snell et al. 2002). Though the population increase was initially—and still primarily is—fueled by ecotourism, the economic pull of the islands' booming fisheries has also drawn people to the archipelago (Boersma et al. 2005; Cohn 1996). The high annual growth rate of 6.0% (1982-1998), compared to only 2.1% on mainland Ecuador, prompted The Special Law for the Galápagos in 1998, which excluded even Ecuadorians from moving to the Galápagos unless they were born there or had family there (Bensted-Smith et al. 2002;

Bremner and Perez 2002; Tanner and Perry 2007). In 2005, the population of the Galápagos exceeded 27,000 people on 4 islands (Boersma et al. 2005).

Roads also pose a great risk to native species. Researchers have observed a reduction in lava lizard abundance near roads and approximately 4,000 birds comprising 19 species were reported killed during a 1-year period on the 40 km road between Puerto Ayora, the largest city in the Galápagos, and the Itabaca Canal on Isla Santa Cruz (Tanner and Perry 2007). During a 10-year period, researchers documented 190 tortoises dead from anthropogenic causes throughout the archipelago, either as a result of the local market for tortoise meat or even a backlash caused by resentment towards the Galápagos National Park (GNP) and conservation regulations in general (Marquez et al. 2007).

Tourism

Eco-tourism is an economic driving force on the islands. Organized cruise boat tourism began in 1969 (Gonzalez et al. 2008). Tourism to the Galápagos Islands is capped at 120,000, but averages 100,000 visitors per year and generates $150 million annually (Boersma et al. 2005). This number of visitors, nearly 5 times the current population, can have dramatic impacts on the ecosystem and despite, or because of the, attractive flagship species, ecotourism in the Galápagos Islands has become unsustainable (Kruger 2005).

Tourism, no matter how well managed, can have disastrous effects for the native wildlife. In New Zealand, the endangered endemic Yellow-eyed penguin (*Megadyptes antipodes*) is a key species for wildlife tourism. Consequently, stress induced

corticosterone concentrations were higher at tourist-exposed sites and corresponded with lower fledging weights and reduced juvenile survival (Ellenberg et al. 2007). While adult hoatzin birds (*Opisthocomus hoazin*) had become habituated to tourist presence, chick survival was lower and juvenile stress response higher in tourist-exposed nests than in undisturbed nest sites in Cuyabeno Reserve, Ecuador (Mullner et al. 2004). The mere transport of people and goods to the islands also poses a threat. For example, the greatest risk of West Nile Virus introduction by mosquitoes to the Galápagos Islands is via airplanes (Kilpatrick et al. 2006). With the steady increase of tourism to the islands, the risk of pathogen introduction is likely to increase as well.

INTRODUCED SPECIES

Humans have introduced hundreds of non-native species to the islands. Several of these species have become invasive and threaten the native biodiversity of the islands. For example, goats and pigs were left on several islands by buccaneers and whalers for food stock for future visits, feral cats are escaped pets, and invasive rodents arrived as stowaways on the numerous ships to the islands (Guo 2006).

By 2001, 45% of alien plant species had naturalized on the islands (Magee et al. 2001). It is estimated the introduced *Wasmannia* ant, which has devastated populations of native ants and has now colonized Santa Cruz, San Cristobol, Isabela, Floreana, Santiago, Santa Fe, Pinzon, and Marchena, was introduced to the islands 35-70 years ago (Causton et al. 2005; Clark and Donoso 1982).

The invasive herbivores (feral goats and pigs) not only destroy the vegetation, but remove the foliage that shades ephemeral rain pools, which regulate temperatures critical to reptile egg development (Kaiser 2001). In the late 1980s, El Niño induced vegetation changes allowed a boom in the goat and pig populations (Guo 2006). The feral goats on Isla Isabela have denuded the island of its native vegetation, the food source for Isabela's local population of 3,000 giant tortoises (Kaiser 2001). Though pigs are primarily herbivorous, they feed opportunistically on animal items, such as eggs and hatchlings of green sea turtles (*Chelonia mydas*) giant tortoises (*Geochelone elephantopus*) and dark rumped petrels, (*Pterodroma phaeopygia*; Coblentz and Barber 1987; Kaiser 2001). The extinction of the land iguana (*Conolophus subcristatus*) on Santiago is attributed to the introduction of feral pigs (Coblentz and Barber 1987). Donkeys, which were first introduced to the Galápagos Islands in 1834 (on Isla Floreana) as pack animals, have impacted the native plant species through grazing and the tortoise and land iguana populations by trampling nests and perhaps through direct competition for resources (Carrion et al. 2007).

The pan-global invasive species *Rattus rattus*, *R. norvegicus*, and *Mus musculus* have been introduced to several islands within the archipelago through the establishment of human settlements and increased traffic from mainland Ecuador (Dowler et al. 2000). *Rattus rattus* (the black rat, ship rat, or roof rat) was the first invasive rodent species introduced to the archipelago likely on whaling boats or pirate ships in the 1600's. The black rat has been recorded on Santa Cruz, Baltra, Floreana, Santiago, Pinzon, San Cristobol, and Isabela as well as several small islets (Clark 1980). The introduction of *R.*

rattus is thought to have contributed to the extinction of *Aegialomys galapagoensis* on San Cristobal. *Mus musculus* was introduced after *R. rattus* although the exact sites and dates of introduction are not known (Key and Heredia 1994). The most aggressive of the introduced rodents, *R. norvegicus*, was first identified on Isla Santa Cruz in 1984 and by 1993 had expanded its range throughout several of the islands (Key and Heredia 1994).

On Isabela, rats have ravaged 2 nesting sites of the mangrove finch whose population is estimated under 100 individuals (Guo 2006). Rats and feral cats have decimated populations of the marine iguana (*Amblyrhynchus cristatus albemarlensis*) on Isla Isabela leading to its listing on the World Conservation Union (IUCN) Vulnerable list in 2004 (Guo 2006).

The subsequent extirpations of 3 endemic rodent species have been attributed to invasions of non-native rodents: *Nesoryzomys indefessus* on Isla Santa Cruz and Isla Baltra, *N. darwini* on Isla Santa Cruz, and *Aegialomys galapagoensis* on Isla San Cristobal. The remaining rodent species are believed to be vulnerable as well. Introduced rodents impact the native rodent species both directly, by attacking endemic rodents, and indirectly by competing for resources, altering habitat, and introducing diseases and parasites (Brosset 1963; Snell et al. 2002). Not surprisingly, insular rodents have been the mammal taxon most vulnerable to cat predation. A single feral cat led to the extirpation of the insular endemic *Peromyscus guardia* on Estanque Island in the Gulf of California (Vazquez-Dominguez et al. 2004). The endemic Galápagos rodents may face a similar fate, because they exhibited naivety towards and no avoidance of traps marked with feline scent (Dexter et al. 2004). Feral cats are present on every island

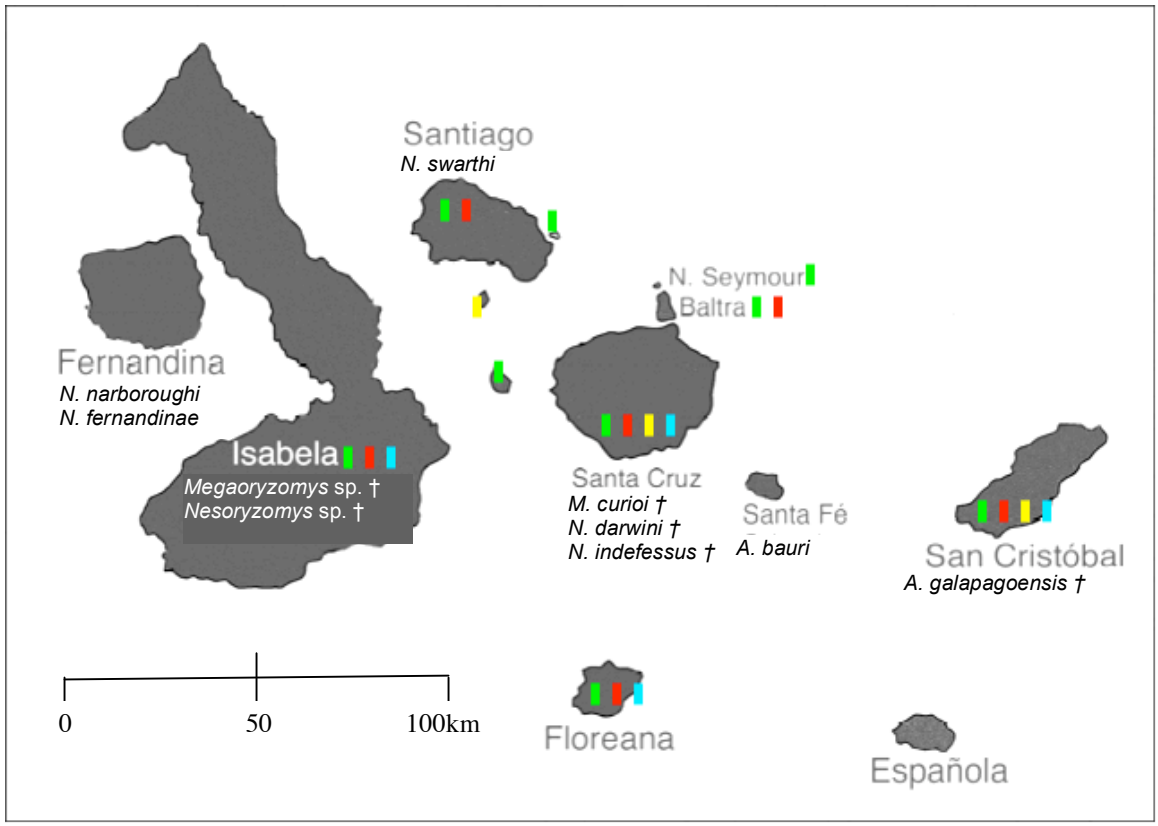

Figure 6. The distribution of introduced species in the Galápagos Islands. Green: *Rattus rattus*, Yellow: *R. norvegicus*, Red: *Mus musculus*, Blue: *Felis catus*. Extinct†. Modified from Dowler et al. 2000.

where a Galápagos native rodent has gone extinct, but have not yet colonized Isla Santiago, Santa Fe, or Fernandina (Fig. 6).

CONSERVATION

To date, 929 introduced plant and animal species have been documented on the Galápagos archipelago (600 plant, 300 invertebrate, and 29 vertebrate species—Snell et al. 2002). There is universal consensus that the native wildlife of the Galápagos Islands should be protected from further anthropogenic endangerment.

The Ecuadorian government has made strides towards conserving the islands' biodiveristy. The Galápagos National Park was established in 1959 and comprises the majority of the land area, 97.5% of the archipelago (Bensted-Smith et al. 2002). There were 1,000-2,000 residents living on 4 islands at the time the GNP was established (Bremner and Perez 2002).

A quarantine inspection system was established in 1999 and subsequently has intercepted 800 prohibited products en route to the Galápagos Islands (Snell et al. 2002). In 2000, the Charles Darwin Research Station and GNP implemented an $18 million eradication initiative, targeting all invasive species, primarily funded by the United Nations and Global Environmental Facility (Guo 2006; Kaiser 2001). In response to international concern, UNESCO added the Galápagos Islands to the list of World Heritage Sites in Danger in 2007.

Eradication Efforts

The eradication of invasive mammals from the Galápagos Islands has been mostly successful. Over 18,000 feral pigs were removed over a period of 30 years on Isla Santiago and the last feral pig was believed to have been killed in April 2000 (Cruz et al. 2005). Feral goats have been eradicated from Isla Isabela, Santiago, and Pinta (Guo 2006). Approximately 140,000 goats have been eradicated from Isla Isabela alone, the largest eradication project undertaken (Guo 2006). Donkeys were opportunistically hunted during feral pig and feral goat eradication efforts using aerial hunting and 339 donkeys were removed over 3 decades on Isla Santiago (Carrion et al. 2007). Survey

data indicate the recovery of the Galápagos rail (*Laterallus spilonotus*) following the eradication of feral pig and goat populations on Isla Santiago (Donlan et al. 2007). After eradications of invasive goats and pigs, researchers have documented an almost immediate rebound by native vegetation. The eradication of feral goats on Isla Pinta resulted in the return of native plants. The island, once grazed by the giant tortoise, of which only Lonesome George remains, is now left without a large herbivore to keep the vegetation in check. Scientists are debating restocking Isla Pinta with another species of captive bred *Geochelone* tortoise (Kaiser 2001). Feral cats were eradicated from Isla Baltra resulting in a rebound in land iguana populations and there are similar implementation plans for Isla Santa Cruz. Invasive rodents continue to be a problem, with only one successful eradication of *Rattus rattus* on Bartolome (Clark 1980).

Status of the Native Rodents

Of the 112 evaluated species on the Galápagos, 54 species (48%) are classified at some level of "Threatened" (4 Critically Endangered species, 12 Endangered species, and 38 Vulnerable species; Snell et al. 2002). Of the 8 vertebrate species listed as extinct in the Galápagos Islands, 5 are rodents (*Megaoryzomys curioi, Megaoryzomys* sp., *Aegialomys galapagoensis, Nesoryzomys darwini, N. indefessus)*. The remaining native rodents potentially are in danger of a similar fate. In fact, in 2008, all 4 extant endemic rodent species were listed as Vulnerable on the IUCN's Red List (Tirira et al. 2008a-b; Tirira et al. 2008a-b). The extirpation of the remaining native rodents and subsequent replacement by the invasive rodents in the Galápagos Islands may lead to habitat

alteration or a change in the plant communities of the islands. Also, the native hawks and owls of the Galápagos Islands prey on the native rodents and the potential impact of the replacement of the native prey items by the larger and more aggressive *Rattus* spp. on these birds has not been investigated.

Several researchers have called for a conservation program for the remaining native rodents. Tye and Snell (2002) put forth criteria for monitoring introduced species, measuring changes in abundance or distribution of the native species in relation to introduced species, and tracking the success of mitigation efforts, such as captive breeding and reintroduction programs. Trillmich (1986) advocated the initiation of a captive breeding program of exporting the endemic rodents to zoological gardens. Dowler and Carroll (1996) suggested a semiannual monitoring program to detect the introduction of *Rattus* or *Mus* to Isla Fernandina and supported the development of captive colonies. Key and Heredia (1994) also called for a monitoring system as well as the need to study the ecology of the native rodents. Dowler et al. (2000) proposed a monitoring system, captive breeding program, and emergency action plan should an introduction occur on Isla Fernandina or Isla Santa Fe. As of 2006, both Isla Santa Fe and Isla Fernandina remained free of introduced rodents (Edwards, pers. comm.).

Knowledge of the colonization, migration, risk of extinction, and genetic structure of a species is vital for the development of an effective conservation strategy. Population genetics is a robust tool in studying conservation biology by identifying reproductive isolation, delineating evolutionary units, and use in captive breeding programs (Burns et al. 2003; Fernando et al. 2006; Swinnerton et al. 2004). Given the lack of any population

genetic data, further examination into the genetic structure of these rodents is warranted. This present study uses molecular data (d-loop sequences and microsatellites) to elucidate the levels of genetic diversity and characterize the amount of population genetic structure among the native rodents of the Galápagos Islands. It is the first to examine all 4 extant endemic rodent species (*N. swarthi*, *N. narboroughi*, *N. fernandinae*, and *A. bauri*) and the first study to use microsatellite data of the endemic species to elucidate population structure.

OBJECTIVES

The objectives of this study were to:

I. Generate mtDNA d-loop sequence data to obtain an estimate of genetic diversity and population genetics parameters for *Nesoryzomys swarthi*, *N. narboroughi*, *N. fernandinae*, and *Aegialomys bauri*. These parameters include haplotype distribution and frequency, haplotype diversity, nucleotide diversity, F_{ST}, and the amount of genetic diversity attributable among and within populations.

II. Generate microsatellite genotype data to obtain estimates of genetic diversity and population genetic parameters of *Nesoryzomys swarthi*, *N. narboroughi*, *N. fernandinae*, and *Aegialomys bauri*. These parameters include allele frequency, observed and expected heterozygosity, polymorphic informative content, F_{ST}, R_{ST}, and other measures of diversity.

III. Compare the estimates of genetic diversity and population genetic parameters to reconstruct evolutionary relationships among lineages and obtain resolution at both the individual and maternal lineage levels.

HYPOTHESES

Aegialomys bauri

I expect *Aegialomys bauri* to exhibit the lowest levels of genetic diversity in d-loop sequence data and microsatellite data as a result of small island size (Isla Santa Fe). Levels of genetic and allelic diversity are related to island size, in that larger islands can sustain larger populations (Hinten et al. 2003; Vega et al. 2007). I expect low observed heterozygosity (H_O) values, low levels of allelic richness (R_G), low percentage of polymorphic loci (P), moderate to high F_{ST} values, and high inbreeding coefficients.

Nesoryzomys swarthi

I expect populations of *Nesoryzomys swarthi* to exhibit low levels of genetic diversity in d-loop sequence data and microsatellite data as a result of genetic bottleneck events and inbreeding. This species is currently sympatric with invasive rodents on Isla Santiago and is restricted to the northern side of the island. I expect low observed heterozygosity (H_O) values, low levels of allelic richness (R_G), low percentage of polymorphic loci (P), moderate to high F_{ST} values, and moderate to high inbreeding coefficients.

Nesoryzomys fernandinae and *Nesoryzomys narboroughi*

I expect *Nesoryzomys fernandinae* and *N. narboroughi* to exhibit moderate or high levels of genetic diversity in d-loop sequence data and microsatellite data (compared to the other endemic species of study) as a result of larger island size (Isla Fernandina) mitigating the effects of genetic drift and the lack of invasive mammals. I expect moderate or high observed heterozygosity (H_O) values, moderate or high levels of allelic richness (R_G), moderate percentage of polymorphic loci (P), low to moderate F_{ST} values, and low to moderate inbreeding coefficients. These two species are sympatric along most of their range. *Nesoryzomys narboroughi* is found in greater abundance along the coast while *N. fernandinae* is found in greater abundance in higher elevations. The elevation of Isla Fernandina ranges from 5m to 1400m. If niche competition results in genetic divergence, then the sympatric *Nesoryzomys fernandinae* and *N. narboroughi* may each exhibit a clinal variation in genetic diversity within the species as a result of differences in abundance.

Nesoryzomys and *Aegialomys*

I expect *Aegialomys bauri, Nesoryzomys swarthi, N. fernandinae,* and *N. narboroughi* to exhibit strong genetic divergence among species. If the time of divergence for the three *Nesoryzomys* species is very recent, then haplotype data may not be able to distinguish between species.

METHODS

JUSTIFICATION OF MOLECULAR MARKERS

To test the level of genetic diversity within and among the 4 species of endemic Galápagos rodents, we used single locus genetic markers: microsatellites and mitochondrial control region (d-loop) sequences.

Microsatellites are simple sequence repeats (SSR) or variable nucleotide tandem repeats (VNTR), which are repeating units of nucleotide sequences and differ in the number of repeats. Microsatellites occupy a single locus, can by assayed with polymerase chain reaction (PCR), can be used in allele genealogy, have many loci available for study, and have high overall variability of length. The rate of mutation is typically 10^{-3} or 10^{-4} per generation or one mutation every 1,000 to 10,000 generations (Allendorf and Luikart 2007). Finally, microsatellites are bi-parentally inherited, are co-dominant, and highly polymorphic. Using several unlinked microsatellite loci minimizes the stochastic variations inherent to a single locus (Sunnucks 2000).

Microsatellite primer pairs developed in one species often can be used in closely related species, because primer sites are generally highly conserved (Allendorf and Luikart 2007). Microsatellite loci have been used successfully in studies of rodent population genetics (Castleberry et al. 2002; Chirhart et al. 2005; Hinten et al. 2003),

however, microsatellite data are not easily comparable between studies. It is important to note that selecting only the most polymorphic markers in the genome, however, causes an ascertainment bias, which may lead to an underestimate of genetic diversity (Väli et al. 2008). Single nucleotide polymorphisms (SNPs) result in higher population diversity estimates than microsatellite heterozygosity (Väli et al. 2008). While sequence based approaches such as SNPs provide more precise indications of genomic diversity, microsatellite markers are still informative in estimating population substructure.

Mitochondrial sequence data are single locus, can be assayed with PCR, can be used in allele genealogy, can be compared to other studies, and ranges in its variability depending on the region of mtDNA sequenced. The control region, or d-loop, exhibits a rate of sequence evolution 4 times that of protein coding regions (Pesole et al. 1999). D-loop sequences define broad lineages or genetic subgroups delineating deeper nodes in the phylogenetic tree and d-loop sequencing has been used in several comparable studies of mammals (Matocq 2002; Mendez-Harclerode et al. 2005; Moritz et al. 1987; Rodriguez and Ammerman 2004).

POPULATION GENETICS

Sample Collection

Tissue samples of *N. fernandinae* (n= 18), *N. narboroughi* (n= 22), *N. swarthi* (n= 17), and *A. bauri* (n= 24) were obtained via loan from the Angelo State Natural History Collections (ASNHC; Appendix Table 15). Additional sampling by Cody W. Edwards and Robert C. Dowler was conducted in the Galápagos Islands in July-August 2006 (Fig.

7; for specific sampling localities see Appendix Figs. 24-26). Genetic material (buccal swabs, fecal pellet, and/or ear biopsy) was collected from captured individuals. A total of 156 samples were collected during this expedition: *N. swarthi* (n= 45), *N. fernandinae* (n= 23), *N. narboroughi* (n= 50), and *A. bauri* (n= 38). High quality DNA from fecal pellets could not be successfully isolated and only tissue samples were included in this study. Total number of samples included in this research was N= 227 (*N. swarthi* n= 56, *N. fernandinae* n= 40, *N. narboroughi* n= 72, and *A. bauri* n= 59).

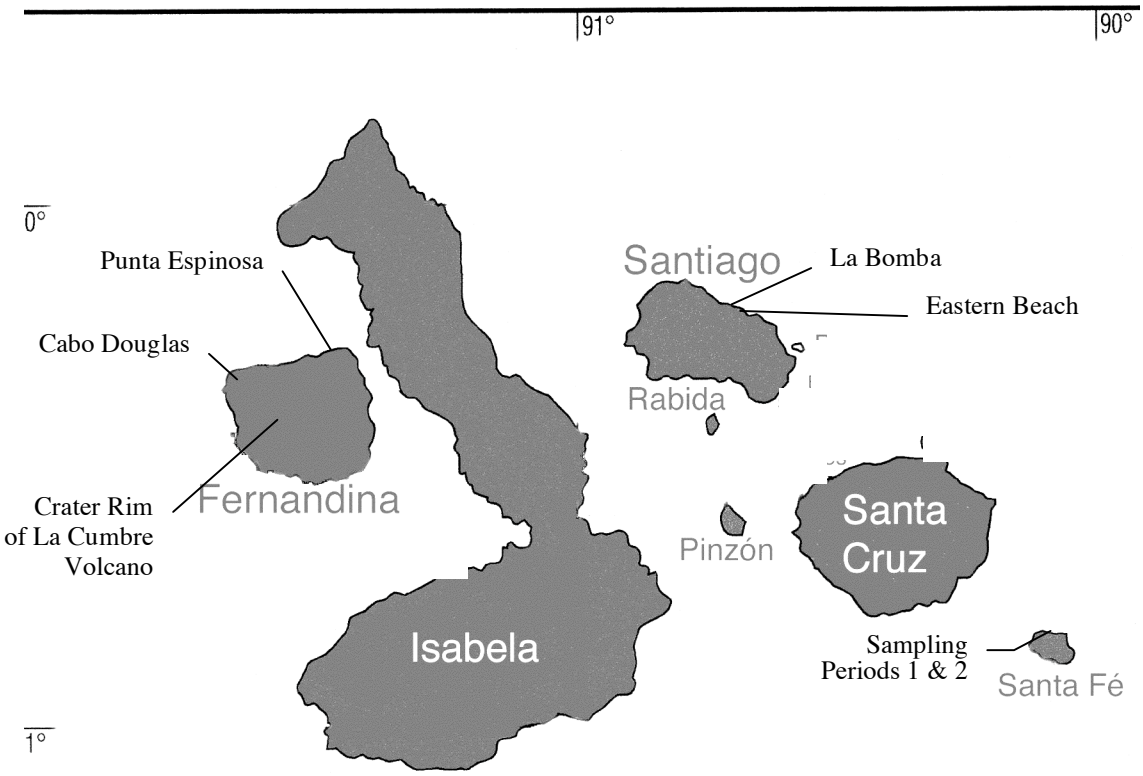

Figure 7. Sampling locations of endemic rodents. *Nesoryzomys fernandinae* was collected from Cabo Douglas and Crater Rim only.

DNA Isolation

DNA was extracted from liver samples (n= 78) and ear biopsies (n=149) using the DNeasy® Blood and Tissue Kit (QIAGEN, Inc., Valencia, CA) by cutting ~20μg of tissue into several small pieces and following manufacturer's protocol. DNA isolations were quantified on a 1% agarose gel using gel electrophoresis (200V, 150mA for 40 minutes). Buccal swabs and fecal pellets were not processed for use in this study.

Microsatellite Protocols

Based on the results of Weksler (2003) and given the lack of previous molecular research involving the native rodents of the Galápagos Islands, I used microsatellite primers designed for *Nectomys squamipes* (Almeida et al. 2000; Maroja et al. 2003). A total of 12 microsatellite loci (Appendix—Table 17) were screened. To ensure proper amplification and specificity, each microsatellite primer set was screened using a small number of samples of each of the 4 endemic rodent species. This screening was conducted across a range of annealing temperatures using a thermal gradient PCR machine (PTC 200™ Gradient Cycler, MJ Research, Inc.) or iCycle (Bio-Rad Laboratories, Inc.). To test for efficacy of polymerase, primers were also screened using 2 types of *Taq* polymerase (AmpliTaq Gold™ or AmpliTaq®, Applied Biosystems, Foster City, CA). The effect of concentration of dNTPs and MgCl and number of cycles (30-45 cycles) were also tested in optimization trials.

Microsatellite reactions consisted of either 8.9 μL diethylpyrocarbonate (DEPC) H_2O, 2.0 μL 10X PCR Gold buffer, 2.0 μL Mg++ mix (25mM), 2.0 μL deoxynucleotide

triphospates (dNTPs, 2mM each), 1.0 μL each forward and reverse 10 μM primer, 1.0 μL betaine, 0.1 μL AmpliTaq-Gold™ DNA polymerase, or 10.9 μL DEPC H_2O, 2.0 μL 10X PCR buffer, 2.0 μL dNTPs, 1.0 μL each forward and reverse 10 μM primer, 1.0 μL betaine, 0.1 μL AmpliTaq® DNA polymerase and 2 μL of DNA for a final reaction volume of 20 μL. Betaine was added to reduce microsatellite stutter in the PCR reaction and minimize scoring errors (Henke et al. 1997; Rees et al. 1993).

Once the ideal PCR procedure was optimized, the forward primer was modified with FAM™ fluorescent labels. Basic amplification procedures were as follows: initial step of 95ºC (11minutes for AmpliTaq Gold™ or 5 minutes for AmpliTaq®), 34 cycles denaturing at 95ºC (30 sec), optimized annealing temperature (30 sec), extension of 72ºC (1.5 minutes at 2 sec/cycle), and a final step of 72ºC (35 min). Negative and positive controls were included with all PCR reactions. The positive control was a random sample that had successfully amplified with a given primer in a previous reaction.

Amplified products were quantified on a 1.5% agarose gel then diluted as needed with DEPC H_2O (1:10, 1:15, or 1:20) based on the intensity of the band when viewed under ultraviolet light. After dilution, 1.2 μL of product were added to 5 μL of HiDi formamide and internal size standard, Internal Lane Standard (ILS) 600 (1:20 solution of ILS to HiDi). The mixture was heat denatured for 3 min at 95ºC then subjected to capillary electrophoresis using SCE 9610 Genetic Analysis System (SpectruMedix, State College, PA).

Running replicates of each sample for microsatellite loci was not necessary because of the high throughput DNA isolated from tissue (versus hair or feces). Any rare

alleles missed due to drop out would yield scoring errors that underestimate allelic diversity. Also, since tissue samples were used (liver and ear biopsies), there is only 1 sample per individual and thus no risk of re-sampling the same individual. Each 96-well plate contained a constant positive control during the analysis of FAM™ labeled loci (i.e. a replicate of a randomly chosen individual was analyzed on each plate of that particular locus). Samples with suspect peak patterns were re-amplified and reanalyzed. Once analyzed by GenoSpectrum, each sample was visually inspected and alleles were called *by eye* to reduce scoring error resulting from mistyping of stutter peaks. Fragment size data was obtained, then binned and scored. A custom PERL (Practical Extraction and Reporting Language) script developed by P.M. Gillevet (GMU) was used to convert raw data files from text files to an excel file for editing and formatting for use with analysis software.

Microsatellite Analyses

Descriptive locus statistics used in this research included the number of polymorphic loci (*P*), allele frequencies, number of alleles (N_A), allelic richness or the number of alleles standardized to the smallest sample size in the study (R_G), and tests for linkage disequilibrium (*LD*). All locus statistics were generated using FSTAT version 2.9.3 (Goudet 2001). In this study, a locus was considered to be polymorphic when the frequency of the most common allele was less than 0.95. The proportion of polymorphic loci (*P*) is the number of polymorphic loci divided by the total number of loci tested, and was included for historical purposes. The number of alleles per locus (N_A) is strongly

dependent on sample size. Allelic richness (R_g) is more informative than N_A as it uses rarefaction methods to estimate allelic richness at a locus for a fixed sample size and is more sensitive to loss of genetic variation (alleles) in small populations than heterozygosity (Allendorf and Luikart 2007). Linkage disequilibrium is a measure of the deviation from random association between alleles at different loci (Hedrick 2005b).

The frequency of private alleles and the number of migrants (*Nm*) were calculated using GENEPOP 3.4 (Raymond and Rousset 1995). The null allele frequency (*r*) for loci having 3 or more alleles was also calculated in GENEPOP 3.4. The null allele frequency for loci having only 2 alleles was calculated using Brookefield's (1996) method in which $r = H_E - H_O / 1 + H_E$. Null alleles fail to produce a visible product during amplification, typically due to a mutation at the priming site, and bias allele frequencies and decrease observed heterozygosity (DeWoody et al. 2006).

A deviation from Hardy-Weinberg Equilibrium (HWE) is one of the first steps in the study of population structure. Tests for HWE per population and θ_H under the infinite allele mutation model (IAM) were calculated using Arlequin ver. 3.0 (Excoffier et al. 2005). The effective population size (N_e), the size of the ideal population (*N*) that will result in the same amount of genetic drift as in the actual population being considered, was calculated using $\theta_H = 4N_e\mu$ (Allendorf and Luikart 2007).

Nei's statistics, including overall gene diversity corrected for sample size (H'_T), amount of gene diversity among samples (D'_{ST}), heterozygote deficit among populations— an F_{ST} analog (G_{ST}), heterozygote deficit within populations (G_{IS}), and estimates corrected for sample size (G'_{ST}), were also calculated (Nei 1978; Nei 1986).

All aforementioned statistics as well as Weir and Cockerham's parameters for F-statistics: F (F_{IT}), θ (F_{ST}), and f (F_{IS}), and Slatkin's R_{ST} per locus and over all loci as well were calculated using FSTAT version 2.9.3 (Goudet 2001).

Wright's F coefficients allocate the genetic variability to the total population level (T), subpopulations (S), and individuals (I). The F_{ST} is a measure of the genetic differentiation over subpopulations and is always positive, ranging from 0 to 1. F-statistic values ranging from 0 to 0.05 represent little genetic differentiation, 0.05 to 0.15 indicates moderate genetic differentiation, 0.15 to 0.25 shows great genetic differentiation, and values greater than 0.25 indicate very great genetic differentiation (Balloux and Lugon-Moulin 2002). F_{IT} is the inbreeding coefficient of individual (I) to the total population (T). F_{IS} is the inbreeding coefficient of individual (I) to a subpopulation (S). Positive F_{IS} and F_{IT} values indicate a deficient of heterozygotes and negative values indicate an excess of heterozygotes.

There are several analogs to Wright's fixation statistic. Weir and Cockerham's (1984) F parameters (F, f, and θ) are unaffected by number of alleles per locus, number of samples per population, or number of populations. Weir and Cockerham's θ is the least biased estimator of F_{ST} (Goudet et al. 1996). For clarification, Weir and Cockerham's analog to F_{ST} will be noted as θ_{WC}. Though Wright initially defined the parameters for F-statistics based on 2 alleles, Nei (1973 and 1977) extended the method to 3 or more alleles with the parameters G_{IS}, G_{IT}, and G_{ST}. Like F_{ST}, G_{ST} can range from 0 to 1, but values tend to be low when using highly polymorphic loci. Slatkin's R_{ST} statistic is unbiased with regard to both sample size between populations, takes into account allele

size, was designed to fit the generalized stepwise mutation model (Slatkin 1995). Hedrick (2005a) presents a standardized measure of genetic differentiation, ranging from 0 to 1, which represents the proportion of the maximum differentiation possible for the level of subpopulation homozygosity observed. Hedrick's standardized measure G'_{ST} will be noted as G''_{ST} in this study to differentiate it from Nei's G'_{ST}, a redefined G_{ST} value that is independent of the number of subpopulations. Hedrick's G''_{ST} allows for a more informative comparison between loci with different mutation rates and between different organisms.

The probability of identity (P_{ID}, the probability that two individuals drawn at random from the same population will have the same genotype) was calculated using IDENTITY 1.0 (Wagner and Sefc 1999). Tests for recent bottleneck events were conducted using the sign test under 3 models of mutation: infinite alleles model (IAM), two-phase model (TPM), and stepwise model of mutation (SMM) in BOTTLENECK 1.0 (Cornuet and Luikart 1996).

Under the infinite allele model (IAM) of mutation, each mutation produces a new allele that is different from all existing ones (Kimura and Crow 1964). Under the stepwise mutation model (SMM), mutations change the state of an allele by one step forward or backward with equal probability—the number of alleles can increase, decrease, or remain unchanged (Kimura and Ohta 1978). The two-phase model (TPM) of mutation, consists of a proportion p of single step mutations and the remaining proportion $1-p$ of larger step mutations (Hedrick 2005b). In this study, the proportion of single step mutations in the TPM model was 0.70. In the sign test, the difference of the observed –

expected heterozygosity across all loci is calculated. A positive difference, or heterozygosity excess, should be observed more often in a population with a recent bottleneck (Cornuet and Luikart 1996).

Regardless of the mutation model, however, alleles at low frequency (<0.1) are always expected to be more abundant than alleles at intermediate (0.1–0.2) or high frequency (Luikart et al. 1998). When graphing the allele frequency classes of microsatellite loci in a population, the distribution of a non-bottlenecked population will exhibit an L-shaped distribution, exhibiting a greater number of alleles in low frequency, with a decrease in the number of alleles as the frequency class increases. Bottlenecks shift the mode from low frequency to an intermediate frequency. This method of testing for recent bottlenecks (within the past few dozen generations) does not require information on historical population sizes or levels of genetic variation, and can be tested on samples of 5 to 20 polymorphic loci and approximately 30 individuals (Luikart et al. 1998; MacAvoy et al. 2007).

Finally, microsatellite data was analyzed in *structure* 2.2.3 (Pritchard et al. 2000) for population differentiation and assignment using 10,000 burn in replicates and 1,000 Markov Chain Monte Carlo replicates after burn in, assuming admixture and correlated allele frequencies to generate a *Q*-plot of population parentage. Each individual is represented by a vertical bar that depicts its estimated membership coefficient, *Q*. Although analysis of *K*=1 to 8 were conducted, only the *Q*-plots based on the *a priori* subpopulation number (*K*) of each species are presented herein.

In summary, the statistics and genetic indices calculated from microsatellite data included: number of polymorphic loci (*P*), number of alleles (N_A), allele frequencies, allelic richness (R_G), tests for linkage disequilibrium (*LD*), Nei's statistics including overall gene diversity corrected for sample size (H'_T), amount of gene diversity among samples (D'_{ST}), heterozygote deficit among populations and corrected for sample size (G_{ST} and G'_{ST}), heterozygote deficit within populations (G_{IS}), Weir and Cockerham's parameters for *F*-statistics: F (F_{IT}), θ (F_{ST}), and f (F_{IS}), and Slatkin's R_{ST} per locus and over all loci were calculated in FSTAT version 2.9.3 (Goudet 2001). Population pairwise F_{ST}, tests for HWE per population, and θ_H under IAM were calculated using Arlequin ver. 3.0 (Excoffier et al. 2005). The frequency of private alleles, number of migrants (*Nm*), and frequency of null alleles (*r*) per population were calculated using GENEPOP 3.4 (Raymond and Rousset 1995). Number of shared microsatellite haplotypes and the probability of identity (P_{ID}) were calculated using IDENTITY 1.0 (Wagner and Sefc 1999). Sign tests and allele frequency distributions were conducted using BOTTLENECK 1.0 (Cornuet and Luikart 1996).

D-loop Sequence Protocol

DNA was amplified using d-loop primers designed for *Oligoryzomys* spp. (Gonzalez-Ittig et al. 2002). A larger final reaction volume was used due to the larger fragment size targeted. PCR conditions consist of 14.85 μL DEPC H_2O, 3.0 μL 10X Reaction buffer, 3.0 μL deoxynucleotide triphospates (dNTPs, 2 mM of each), 1.5 μL each forward and reverse 10 μM primer, 3.0 μL of 0.1% bovine serum albumin (BSA),

0.15 μL AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA), and 3 μL of DNA for a final reaction volume of 30 μL. Bovine serum albumin (BSA) is effective in accommodating various inhibitors of PCR and prevents lipids and anions from binding to and inactivating *Taq* polymerase (Kreader 1996). Since all samples were lysed with protease K, both endogenous and added protease are possible sources of inhibition. BSA relieves inhibition from samples containing endogenous protease activity (Kreader 1996).

Amplification parameters were as follows: initial step of 95ºC (4 min), followed by 34 cycles of denaturing at 95ºC (40 sec), 50ºC (30 sec), extension of 72ºC (2 min at 5 sec/cycle), and a final step of 72ºC (10 min). Reaction products were purified to remove primer fragments using AMPure Magnetic Beads (Agencourt Bioscience, Beverly, MA). The *Oligoryzomys* d-loop primers were used in an initial sequencing run. More specific internal forward and reverse primers were designed using the sequence fragments obtained from this initial run. The internal primers GIF and GIREV were used in all sequencing reactions (Appendix—Table 16). Five microliters of cleaned PCR product were added to 4μL of ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA) and 1.0 μL of 1.6 μM internal primer in each sequencing reaction. Sequencing reaction conditions were: 96ºC (1 min), 45 cycles of 96ºC (30 sec), 58ºC for GIF or 52ºC for GIREV (15 sec), 60ºC (4 min), followed by a final holding step of 4ºC. All sequencing reactions were performed using GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA). Final sequencing products were purified using Sephadex G-50 powder then dried in a vacuum centrifuge and stored at 0ºC. Sequences were re-hydrated with the addition of 5μL of HiDi Formamide with

0.1 mM EDTA, denatured at 95ºC (3 min) and sequenced with capillary action electrophoresis using SCE 9610 Genetic Analysis System (SpectruMedix, State College, PA).

D-loop Sequence Analyses

Sequence data was edited and aligned *by eye* using Sequencher™ 4.1 software (Gene Codes Corp., Ann Arbor, MI). Alignments were verified using Clustal X (Larkin at el. 2007 and Thompson et al. 1997) and JalView 2.0 (Waterhouse et al. 2009).

Sequence composition, number of polymorphic sites (*S*), haplotype frequencies, gene (haplotype) diversity (*h*), nucleotide diversity (π), and an analysis of molecular variance (AMOVA) using 1,000 permutations to estimate Φ_{ST} (an analog of F_{ST}) were calculated with an allowed level of missing data of 5% in Arlequin. Gene or haplotype diversity (*h*) is the probability that two randomly chosen haplotypes (a unique nucleotide sequence) are different in the sample (Allendorf and Luikart 2007; Hedrick 2005b). Nucleotide diversity, π, is the probability that two randomly chosen homologous nucleotides in a pair of sequences are different.

Haplotype genealogy networks were produced using TCS 1.21 (Clement et al. 2000), which collapses sequences into haplotypes and calculates their frequencies. Gaps were treated as a fifth state and missing data was coded as "?". Networks were calculated using a 95% connection limit.

The time (in generations), *t*, of a possible population expansion was estimated through $\tau = 2ut$, where τ is the time to expansion based on the mismatch distribution

mode (calculated in Arlequin) and u is the mutation rate of the sequence. The parameter $u = 2\mu k$, with μ the mutation rate per nucleotide and k the number of nucleotides (Rogers and Harpending, 1992). For the d-loop sequences of this study, k varied by species, and the average μ for mammalian d-loop = 0.04 per million years, or 4×10^{-8} per generation (Pesole et al. 1999), if we assume a generation time of one year for oryzomine rodents.

Though population genetic diversity was the primary objective of this study, a basic phylogenetic analysis of d-loop sequences was conducted to confirm the species designation of the 4 taxa. Deletions, transitions, and transversions were weighted equally. Gaps were coded as a fifth state instead of as "missing data." The model of evolution employed was selected using Akaike Information Criterion (AIC) in ModelTest 3.7 (Posada and Crandall 1998). Neighbor-joining (NJ) analyses were generated using PAUP* 4.0 (Swofford 2002), a Maximum Likelihood analysis was conducted in GARLI 0.96 (Zwickl 2008), and a Bayesian analysis was conducted in BEAST 1.4.8 (Drummond and Rambaut 2007) to verify monophyletic assemblages of the 4 endemic rodent species.

RESULTS

Two primers, Nect17 and Nect19, did not amplify samples of *Nesoryzomys* spp. or *Aegialomys bauri* under any conditions. An additional primer, Nect15, amplified *A. bauri* samples, but not *N. fernandinae, N. narboroughi,* or *N. swarthi* samples. One primer, Nect18, amplified all *Nesoryzomys* samples, but did not amplify *A. bauri* samples. All other samples expressed the alleles amplified by the remaining primers: Nect08, Nect12, Nect13, Nect14, Nect23, Nect24, Nect28, and Nect29. Loci Nect13 and Nect28 were monomorphic in all species for an allele at 209bp and 174 bp, respectively. Nect14 was monomorphic for *N. swarthi* and *N. narboroughi*. Therefore, these samples were not included in any subsequent analyses of genetic diversity. Loci Nect23 and Nect29 amplified in all samples, but amplified at several locations producing numerous allele peaks. These loci could not be scored with enough confidence to include them in the analyses.

Some d-loop samples exhibited shadow bands at a smaller size. These bands represented non-target product. The product band corresponding to the expected size for the d-loop was extracted from the agarose gel with a pipette. The extracted product was then re-amplified using PCR until all shadow bands were eliminated. In some cases, these bands could not be eliminated. Since the resulting sequences could represent pseudogenes and not the target d-loop, these samples were removed from the analysis.

PHYLOGENETIC RESULTS

Though phylogenetic analysis was not the primary objective, NJ, ML, and Bayesian analysis was conducted to verify monophyletic assemblages of the 4 endemic species. Bootstrapping was not conducted on NJ and ML analyses, because phylogenetic relationships between species were not within the scope of this project. Low levels of nucleotide diversity precluded meaningful reconstruction within species. All 3 tree-building methods were conducted using *Pseudoryzomys simplex* (GenBank Accession No. AY863422.1) and *Oligoryzomys longicaudatus* (GenBank Accession No. AY007340.1) as the outgroup. The General Time Reversible model of evolution with rate variation among sites (GTR+ G) was used with a gamma distribution shape parameter = 0.755. Base frequencies were: 35% A, 22% C, 15% G, and 28% T. Each taxon was supported as a distinct species in all 3 tree-building methods, but within-species evolutionary relationships were not resolved. The NJ tree placed *N. fernandinae* as a paraphyletic group basal to all endemic species. This is most likely an artifact of the tree building method and resolution of the d-loop. Though paraphyletic, no members of *N. fernandinae* were placed with any other species. Both ML and Bayesian methods yielded monophyletic clades for all species. The Bayesian tree constructed with a 1,000,000 MCMC chain length is the only method presented (Fig. 8)

Aegialomys bauri and *N. narboroughi* were sister taxa in all analyses. In Bayesian analyses, *A. bauri* and *N. narboroughi* formed a clade sister to *N. swarthi* and *N. fernandinae*. *Nesoryzomys swarthi* and *N. narboroughi* grouped as independent

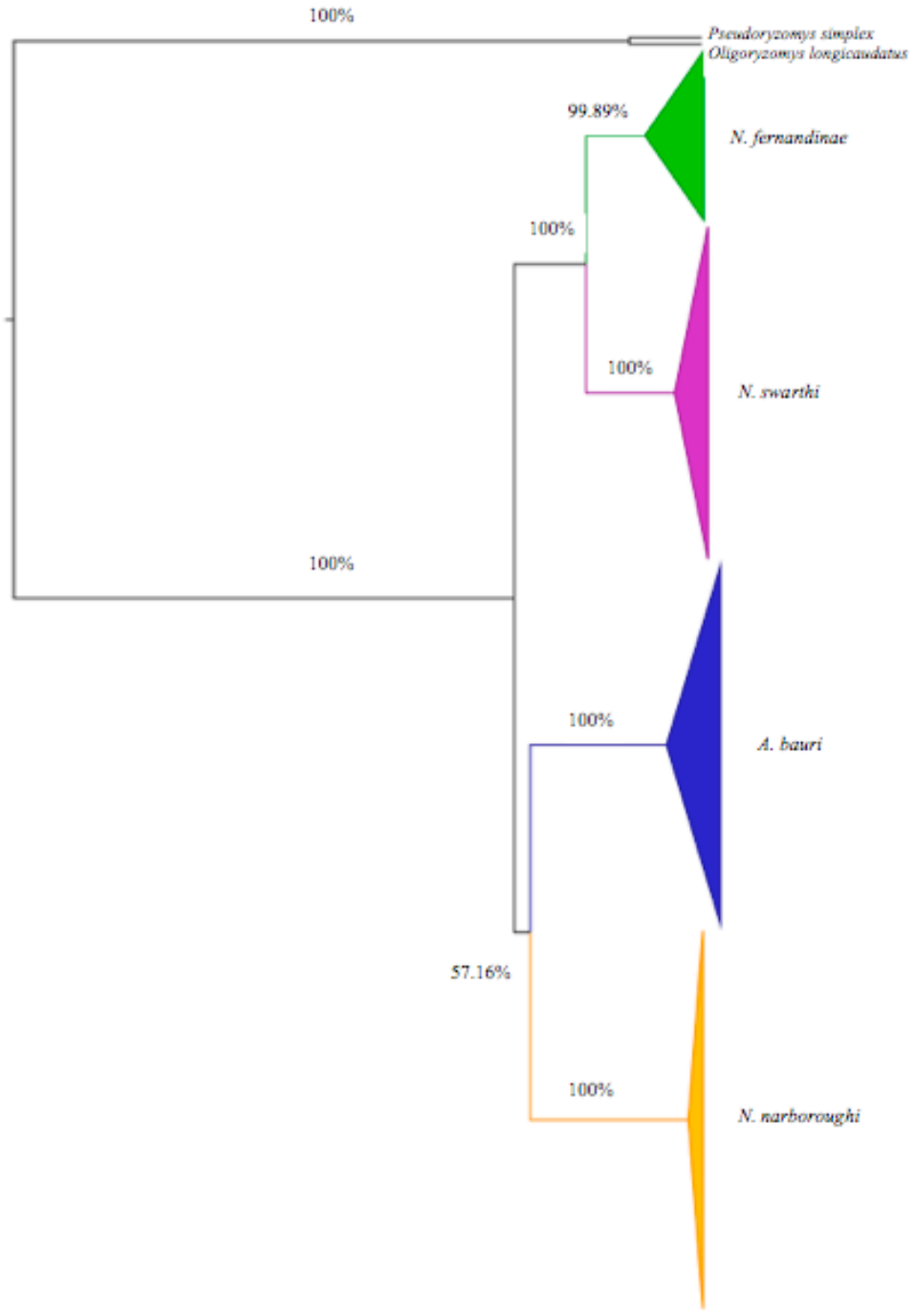

Figure 8. A Bayesian tree containing all extant species of endemic Galápagos rodents. Taxa are color-coded for reference (Blue= *A. bauri*, Yellow= *N. narboroughi*, Purple= *N. swarthi*, and Green= *N. fernandinae*). Outgroup taxa are in red. Posterior probabilities are presented above branches.

clades, contrary to the argument that they are both races of the same species—*N. indefessus* (Patton and Hafner 1983), and gives further support for their species designation. The phylogenetic relationships between species and estimated time of divergence, though interesting, are not within the scope of the current study and more robust analyses are required.

AEGIALOMYS BAURI

Microsatellite Results

Fifty-nine samples of *Aegialomys bauri* were screened for 12 microsatellite loci. Seven loci amplified correctly: Nect08, Nect12, Nect14, Nect15, Nect24 were polymorphic, while Nect18 and Nect28 were monomorphic. The resulting proportion of polymorphic loci was P= 0.417. The number of alleles for *A. bauri* per locus ranged from 4 to 9 (mean N_A= 5.6). The 2 subpopulations represented 2 sampling periods: one from 1997 and 1999 designated Sampling Period 1 (n= 23) and the other from 2006 designated Sampling Period 2 (n= 36; Appendix Fig. 24). Locus Nect14 failed to amplify in any of the samples from Sampling Period 2. Therefore, estimates of population differentiation were conducted using the remaining 4 loci. Linkage disequilibrium was not detected among included loci using 200 permutations and an adjusted p-value for 5% nominal level of 0.005. (Table 2).

Table 2. Tests of linkage equilibrium *p*-values between loci in *A. bauri*. Adjusted significance value = 0.005.

Loci	Sampling Period 1	Sampling Period 2	Pooled *A. bauri*
Nec08 X Nec12	0.550	1.000	0.975
Nec08 X Nec15	0.238	0.725	0.529
Nec08 X Nec24	1.000	1.000	1.000
Nec12 X Nec15	0.300	0.388	0.279
Nec12 X Nec24	0.692	0.188	0.296
Nec15 X Nec24	0.454	0.346	0.363

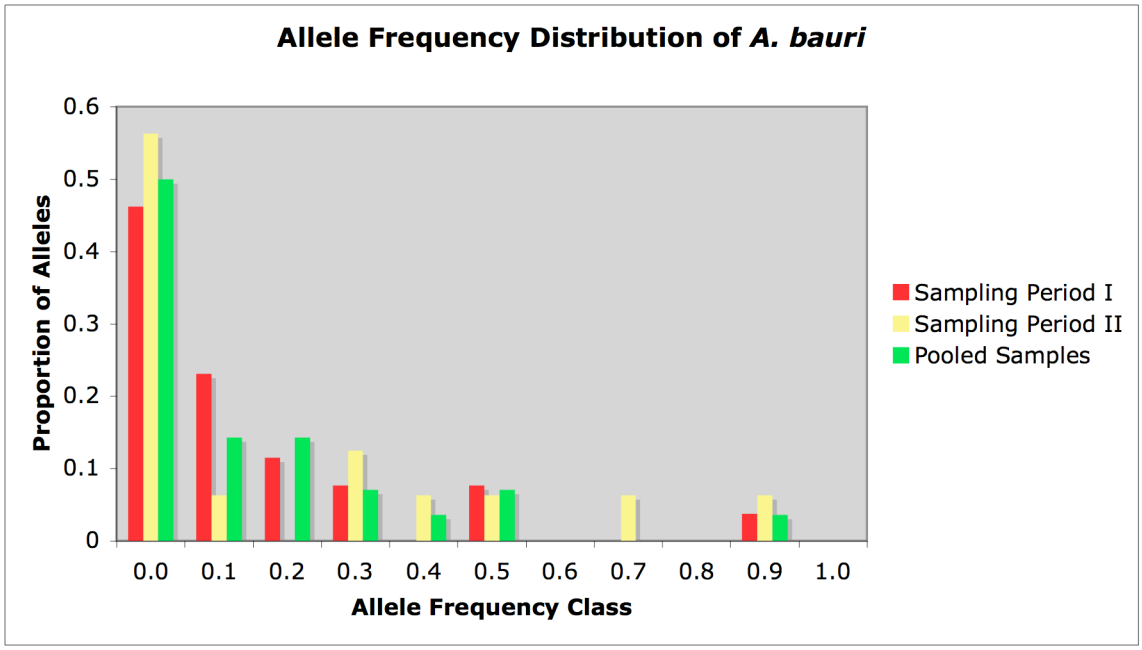

Figure 9. The allele frequency distribution of microsatellite loci in *A. bauri*.

Using the sign test, testing across all loci, we accept the null hypothesis of mutation-drift equilibrium under the IAM, TPM, and the stricter SMM for Sampling Period 1 (p= 0.285, 0.111, and 0.114, respectively), Sampling Period 2 (p= 0.586, 0.544, and 0.181, respectively), and the pooled population (p= 0.3111, 0.3278 and 0.0944, respectively). The alleles frequency distribution exhibits a normal L-shaped distribution under a mode-shift test (Fig. 9).

The program IDENTITY 1.0 (Wagner and Sefc 1999) was used to identify microsatellite haplotypes and probability of identity (P_{ID}). There were 3 shared microsatellite haplotypes, each found only in Sampling Period 2. Haplotype Ob1M is represented by 3 individuals. The 2 other shared haplotypes, Ob2M and Ob3M, are represented by 2 individuals each. The remaining individuals sampled exhibited a unique microsatellite haplotype (Ob4M through Ob52M). The frequency of private alleles was 0.039. Over all 5 loci, the probability of identity for *A. bauri* was 0.00385. The number of migrants corrected for sample size between subpopulations was Nm= 2.867.

Theta-H (θ_H) over loci for Sampling Period 1, Sampling Period 2, and pooled *A. bauri* samples was calculated as θ_H = 1.407, 0.516, and 1.529, respectively. Using this θ_H and a mutation rate μ= 0.0001 for microsatellite loci, the N_e was 3,517 (Sampling Period 1), 1,290 (Sampling Period 2) and 3,822 (pooled *A. bauri*).

When adjusted for missing data, the fixation index, F_{IS}, across all loci for Sampling Period 1 was 0.388 (Table 3). This value indicates a heterozygote deficiency and deviation from Hardy-Weinberg equilibrium. Sampling Period 2 had an overall F_{IS} = -0.047, and a heterozygote excess at 3 of 4 loci (Nect08, Nect12, and Nect15—Table 3).

Table 3. Allelic diversity and deviation from Hardy-Weinberg proportion among microsatellite loci in *A. bauri*. Number of alleles N_A, allelic richness R_G, gene diversity H_S, observed and expected heterozygosity (H_O and H_E), *p*-values for Hardy-Weinberg Equilibrium, and F_{IS} per locus per subpopulations are included. Numbers in parentheses represent sample size.

Locus	Number of Alleles N_a	Allelic Richness R_G	Gene Diversity H_S	Freq of Null Alleles r	Observed Heter. H_O	Expected Heter. H_E	HWE *p*-value	Theta-H θ_H	Fixation Index F_{IS}
Sampling Period 1 (23)									
Nect08 (23)	6	5.809	0.670	0.379	0.217	0.660	<0.0001	1.904	0.676
Nect12 (23)	5	4.675	0.608	0.098	0.522	0.606	0.037	1.537	0.141
Nect14 (16)	4	4.000	0.748	0.126	0.500	0.740	0.011	NA	NA
Nect15 (23)	9	6.178	0.807	0.109	0.609	0.803	0.049	4.074	0.246
Nect24 (17)	2	2.270	0.118	0.243	0.000	0.114	0.032	0.129	1.000
Overall:	= 26	4.733	0.497	0.191	0.369	0.585	<0.0001	1.406	0.388
Sampling Period 2 (36)									
Nect08 (36)	4	3.436	0.393	0.000	0.444	0.394	0.878	0.649	-0.131
Nect12 (36)	5	4.810	0.633	0.000	0.667	0.634	0.872	1.731	-0.053
Nect15 (36)	4	3.850	0.612	0.000	0.639	0.613	1.000	1.582	-0.043
Nect24 (32)	3	2.063	0.063	0.000	0.031	0.062	0.016	0.066	0.500
Overall:	= 16	3.540	0.422	0.000	0.356	0.340	0.335	0.516	-0.047

Tests for HWE were calculated using an exact test with a Marchov Chain of 100,000 and 1,000 dememorization steps, respectively, in Arlequin ver. 3.0 (Excoffier et al. 2005). Sampling Period 1 exhibited heterozygote deficiency across all loci ($p < 0.0001$). Sampling Period 2, however, appeared to be in HWE at 3 of 4 loci analyzed and overall ($p= 0.335$).

Comparisons of various *F*-statistics indicate population substructure between sampling periods in *A. bauri* (Table 4). Slatkin's R_{ST} value indicated only slight differentiation: 0.034 (weighted), 0.029 (Goodman's), and 0.029 (unweighted) (Table 4). However, θ_{WC} (0.158), Nei's G'_{ST} (0.153), and Hedrick's G''_{ST} (0.241) indicated great differentiation and D'_{ST} (0.088) indicated moderate differentiation between Sampling Period 1 and Sampling Period 2 (Table 4; Fig. 10).

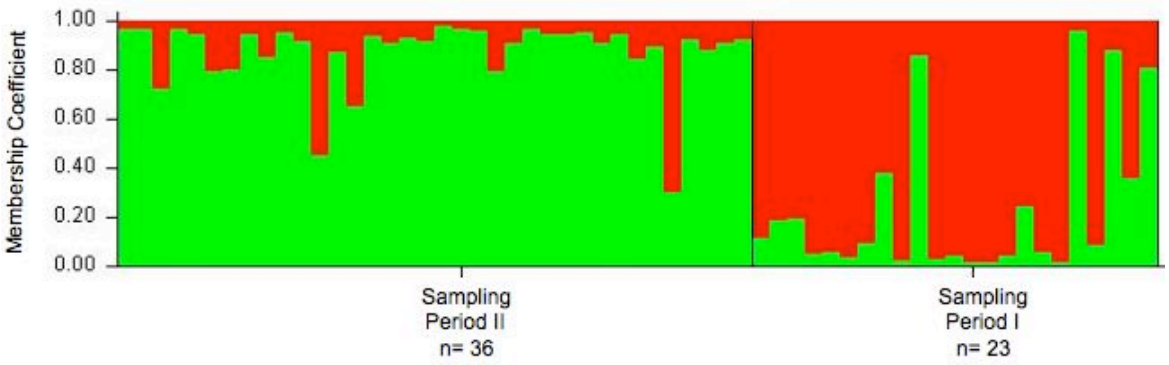

Figure 10. *Structure* Q-plot indicating the level of genetic substructure in *A. bauri* (*K*= 2).

Table 4. Genetic diversity indices per locus for pooled samples of *A. bauri*. Nei's within sample gene diversity or subpopulation heterozygosity (H_S), Nei's overall gene diversity independent of sample size H'_T , Weir and Cockerham's F (F_{IT}), θ_{WC} (F_{ST}), f (F_{IS}), Nei's gene divergence among samples independent of sample size (D'_{ST}), Nei's measure of heterozygosity deficit among populations (G_{ST}) and independent of sample size (G'_{ST}), Hedrick's standardized G_{ST} measure (G''_{ST}), and Slatkin's weighted R_{ST} are included.

Locus	H_S	H'_T	F	θ_{WC}	f	D_{ST}	D'_{ST}	G_{IS}	G_{ST}	G'_{ST}	G''_{ST}	R_{ST}
Nect08	0.530	0.842	0.562	0.386	0.287	0.156	0.313	0.375	0.288	0.371	0.742	0.061
Nect12	0.620	0.641	0.053	0.033	0.021	0.010	0.021	0.042	0.017	0.033	0.072	-0.007
Nect15	0.709	0.728	0.114	0.028	0.088	0.010	0.019	0.120	0.013	0.027	0.076	0.027
Nect24	0.090	0.088	0.746	-0.010	0.749	-0.001	-0.001	0.826	-0.007	-0.015	-0.008	0.035
Overall	***0.487***	***0.575***	***0.281***	***0.158***	***0.147***	***0.044***	***0.088***	***0.197***	***0.083***	***0.153***	***0.241***	***0.034***

D-loop Results

The d-loop was successfully sequenced for 56 individuals of *A. bauri* (Sampling Period 1 n= 20, Sampling Period 2 n= 36). Sequence data was analyzed in Arlequin ver. 3.0 (Excoffier et al. 2005) under the Tamura model with gamma correction α= 0.227. Sequences consisted of 629 bp with 442 bp having less than 5% missing data and 131 polymorphic sites (*S*). One d-loop haplotype, Ob1D, was shared among 4 individuals (1 from Sampling Period 1 and 3 from Sampling Period 2). The haplotypes Ob2D and Ob3D were found only in Sampling Period 2 and were shared by 2 individuals (relative frequencies = 0.056). The remaining haplotypes were each represented by a single individual: Ob4D through Ob22D from Sampling Period 1 (relative frequencies = 0.05) and Ob23D through Ob51D from Sampling Period 2 (relative frequencies = 0.028).

Sequence data obtained for samples from Sampling Period 1 were 464 bp in length with less than 5% missing data and 84 polymorphic sites (*S*). Haplotype diversity was high (*h*= 1.000 ± 0.0158) with low nucleotide diversity (π= 0.00901 ± 0.00523). Nucleotide composition consisted of 27.46% C, 10.47% G, 27.53% T, and 34.54% A with 12 observed transitions, 27 transversions, and 54 insertions/deletions (indels). Sequence data obtained for samples from Sampling Period 2 were 503 bp in length with less than 5% missing data and 119 polymorphic sites (*S*). Haplotype diversity was also high (*h*= 1.000 ± 0.0065) with a nucleotide diversity of 0.0091 ± 0.0051. Nucleotide composition consisted of 27.05% C, 10.51% G, 27.66% T, and 34.78% A with 28 transitions, 24 transversions, and 76 indels.

The haplotype diversity for pooled *A. bauri* was h= 1.000 ± 0.0034. Estimated Φ_{ST} was low and non-significant (0.007 with p= 0.245 ± 0.014). The number of migrants estimated between the two subpopulations was higher than that estimated using microsatellite data (Nm= 71.862).

Complete sequences (629 bp) were used to construct haplotype networks in TCS 1.21. Only haplotypes that could be connected within 10 steps (nucleotide changes) were included by TCS. The resulting haplotype network contained the same haplotype Ob1D present in 4 individuals (2 from Sampling Period 1 and 2 from Sampling Period 2) and Ob2D present in 2 individuals (both Sampling Period 2). The haplotype Ob3D present in 2 individuals was not returned as a common haplotype in analyses using TCS. The haplotype network was highly interconnected with several haplotypes closely related to more than one sister haplotype (Fig. 11). Fifteen haplotypes could not be connected within 10 steps, nor under a lower connection limit of 90%. As a result, the haplotype network included herein (*A. bauri)* does not include these 15 unconnected haplotypes.

The parameters of population expansion based on a mismatch distribution were τ= 4.586, θ_0 = 7.634, and θ_1 = 46.958. If k= 629 nucleotides, then u= 5.032 x 10^{-5}, and using t= τ/ 2u, the time from the most recent population expansion was 45,568 yrs (generations).

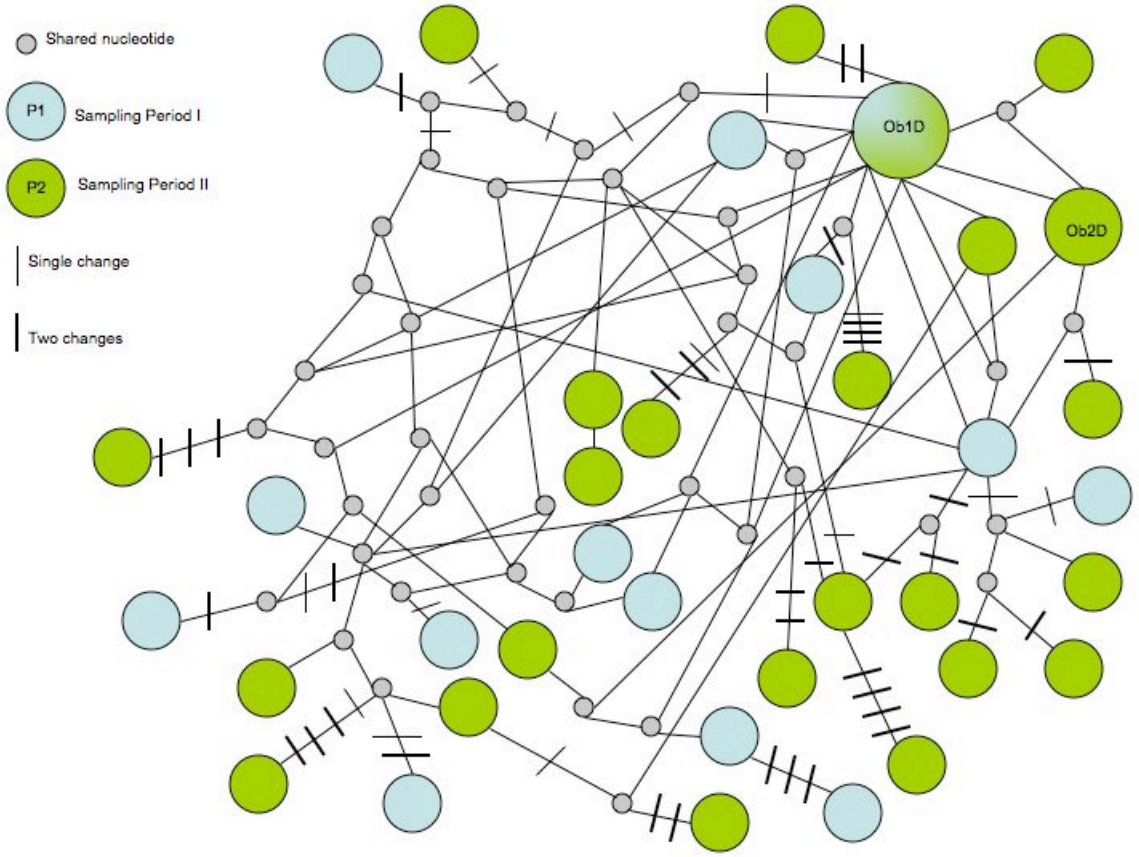

Figure 11. TCS haplotype network for *A. bauri*. Lines connecting haplotypes (circles) represent a single step or nucleotide substitution. Thin hash lines indicate an additional single difference between connected haplotypes and thick hash lines indicate 2 differences. Haplotype Ob1D is present in 4 individuals shared between Sampling Period 1 and Sampling Period 2. Haplotype Ob2D is represeneted by 2 individuals frim Sampling Period 2.

NESORYZOMYS SWARTHI

Microsatellite Results

Fifty-six individuals of *Nesoryzomys swarthi* were collected from 2 locations, La Bomba (n= 38) and Eastern Beach (n= 18). Seven of 12 screened loci amplified product. Five microsatellite loci (Nect08, Nect12, Nect14, Nect18, and Nect24) were polymorphic. The proportion of polymorphic loci for *N. swarthi* = 0.417. The number of alleles ranged from 3 (Nect08) to 12 (Nect24) with the average N_A= 7.8. The mean frequency of private alleles = 0.037. No loci exhibited indications of linkage disequilibrium under 240 permutations with an adjusted *p*-value = 0.004 (Table 5). The frequency of null alleles (*r*) was high for all loci in the Eastern Beach subpopulation (Table 6).

Nine La Bomba samples and none of the Eastern Beach (Appendix—Fig. 25) samples amplified for Nect14. It is worth noting, however, that among the 9 individuals that did amplify for Nect14, 7 alleles were scored. Due to the level of missing data, Nect14 was dropped from analyses of genetic differentiation between localities.

Table 5. Tests for linkage equilibrium *p*-values between loci in *N. swarthi* subpopulations. Adjusted *p*-value = 0.004.

Loci	Eastern Beach	La Bomba	Total *N. swarthi*
Nect08 X Nect12	1.000	0.363	0.638
Nect08 X Nect18	0.579	0.517	0.554
Nect08 X Nect24	0.883	0.988	0.979
Nect12 X Nect18	0.750	0.288	0.346
Nect12 X Nect24	0.288	0.188	0.100

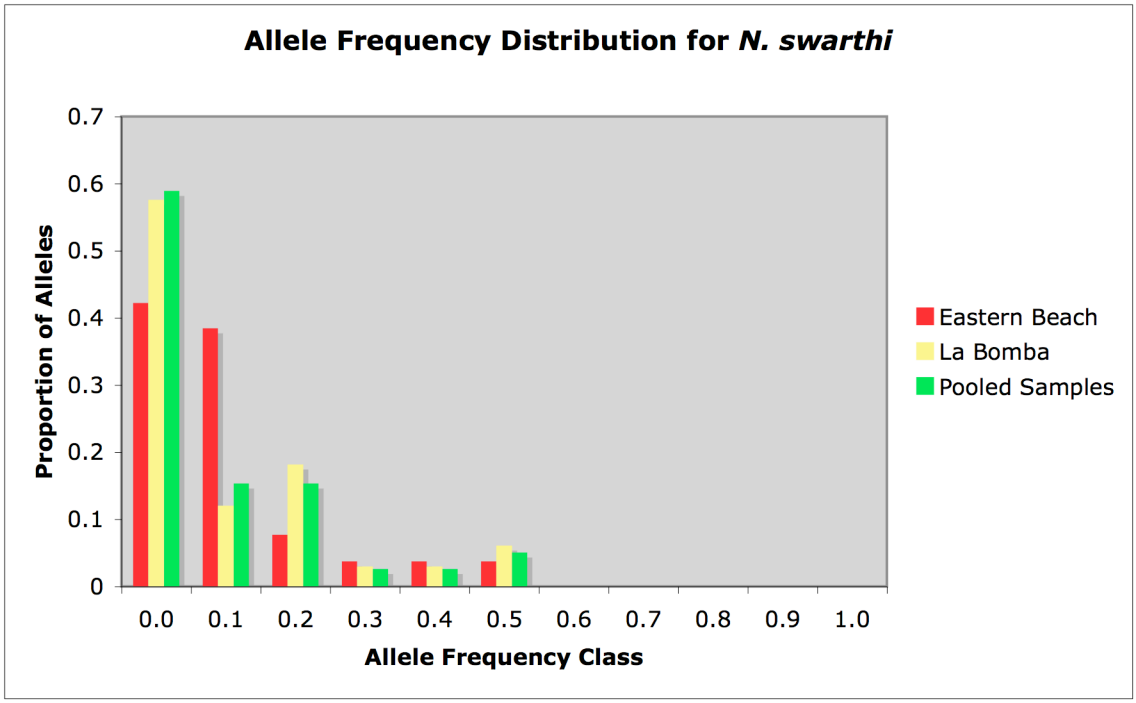

Figure 12. Allele frequency distribution of microsatellite loci in *N. swarthi*.

The P_{ID} across the 5 loci for all *N. swarthi* = 0.00018. Only 1 shared microsatellite haplotype, Ns1M, (shared between 1 female and 1 male in the La Bomba subpopulation) was documented. Neither locality showed evidence of a recent population bottleneck under the 3 models of mutation employed. The sign test *p*-values for IAM, TPM and SMM were 0.444, 0.469, and 0.474, respectively (Eastern Beach locality) and 0.322, 0.661, and 0.334, respectively (La Bomba locality). The allele frequency distributions demonstrated an L-shaped pattern (Fig. 12).

The θ_H for Eastern Beach, La Bomba, and pooled samples of *N. swarthi* was 1.449, 2.596, and 2.023, respectively (Table 6). The N_e for Eastern Beach, La Bomba,

and pooled *N. swarthi* samples was 3,623, 6,489, and 5,056, respectively. The number of migrants estimated between subpopulations after a correction for size was *Nm*= 3.472.

The F_{IS} for Eastern Beach and La Bomba subpopulations across all loci was 0.272 and 0.250, respectively (Table 6). These values indicated heterozygote deficiency and deviation from HWE. The Eastern Beach subpopulation demonstrated deviations from HWE at 2 loci, Nect18 and Nect24, and exhibited strong heterozygote deficiency (p= 0.010 and <0.0001, respectively). The La Bomba subpopulation showed a similar pattern with heterozygote deficiency at Nect08, Nect18, and Nect24 (p= 0.023, <0.0001, and <0.0001, respectively; Table 6).

There was no genetic differentiation estimated between Eastern Beach and La Bomba (θ_{WC} = -0.012, G'_{ST} = -0.012, and G''_{ST} = -0.038; Table 7). Slatkin's R_{ST} across all loci values = -0.0193 (weighted), -0.0222 (Goodman adjusted), and -0.022 (unweighted). Although some genetic variation was documented within the two subpopulations, the Eastern Beach and La Bomba subpopulations show little genetic variation between them (Table 7; Fig. 13).

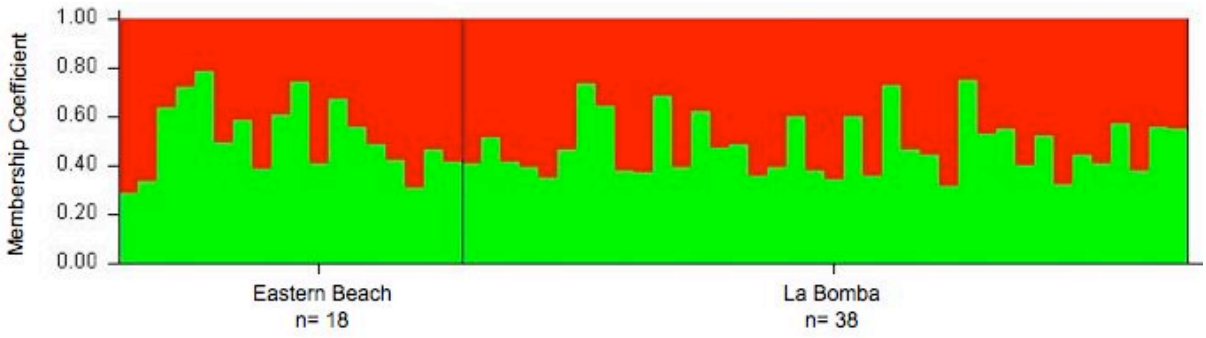

Figure 13. *Structure* Q-plot showing level of genetic substructure in *N. swarthi* (K= 2)

Table 6. Allelic diversity and deviation from Hardy-Weinberg proportion among microsatellite loci in *N. swarthi*. Number of alleles N_A, allelic richness R_G, gene diversity H_S, observed and expected heterozygosity (H_O and H_E), *p*-values for Hardy-Weinberg Equilibrium, and F_{IS} per locus per subpopulations are included. Numbers in parentheses represent sample size.

Locus	Number of Alleles N_a	Allelic Richness R_G	Gene Diversity H_S	Freq of Null Alleles (*r*)	Observed Heter. H_O	Expected Heter. H_E	HWE *p*-value	Theta-H θ_H	Fixation Index F_{IS}
Eastern Beach (18)									
Nect08 (17)	3	3.000	0.586	0.225	0.529	0.585	0.104	1.408	0.097
Nect12 (18)	7	6.889	0.837	0.163	0.889	0.838	0.917	5.176	-0.063
Nect18 (17)	6	6.000	0.649	0.105	0.529	0.645	0.010	1.819	0.184
Nect24 (18)	10	9.886	0.910	0.379	0.222	0.891	<0.0001	8.130	0.756
Overall:	= 26	5.155	0.706	0.217	0.542	0.740	<0.0001	1.449	0.272
La Bomba (38)									
Nect08 (28)	3	2.999	0.569	0.238	0.393	0.566	0.023	1.302	0.309
Nect12 (38)	9	8.190	0.840	0.054	0.868	0.840	0.804	5.264	-0.034
Nect18 (33)	5	4.552	0.609	0.129	0.455	0.607	<0.0001	1.541	0.253
Nect24 (36)	9	7.770	0.825	0.215	0.417	0.819	<0.0001	4.532	0.495
Overall:	= 26	5.878	0.476	0.139	0.560	0.722	<0.0001	2.595	0.250

Table 7. Genetic diversity indices per locus in pooled samples of *N. swarthi*. Nei's within sample gene diversity or subpopulation heterozygosity (H_S), Nei's overall gene diversity independent of sample size H'_T , Weir and Cockerham's F (F_{IT}), θ_{WC} (F_{ST}), f (F_{IS}), Nei's gene divergence among samples independent of sample size (D'_{ST}), Nei's measure of heterozygosity deficit among populations (G_{ST}) and independent of sample size (G'_{ST}), Hedrick's standardized G_{ST} measure (G''_{ST}), and Slatkin's weighted R_{ST} are included.

Locus	H_S	H'_T	F	θ_{WC}	f	D'_{ST}	D'_{ST}	G_{IS}	G_{ST}	G'_{ST}	G''_{ST}	R_{ST}
Nect08	0.578	0.564	0.209	-0.025	0.228	-0.007	-0.014	0.202	-0.012	-0.025	-0.045	-0.016
Nect12	0.838	0.841	-0.040	0.003	-0.043	0.001	0.003	-0.048	0.002	0.003	0.023	-0.009
Nect18	0.629	0.616	0.212	-0.021	0.229	-0.007	-0.013	0.218	-0.010	-0.021	-0.044	-0.033
Nect24	0.866	0.856	0.583	-0.011	0.587	-0.005	-0.110	0.631	-0.006	-0.012	-0.084	-0.031
Overall	***0.728***	***0.719***	***0.247***	***-0.012***	***0.255***	***-0.004***	***-0.009***	***0.261***	***-0.006***	***-0.012***	***-0.038***	***-0.019***

D-loop Results

The d-loop was successfully sequenced in 51 individuals (La Bomba n= 33; Eastern Beach n=18). Sequence data was analyzed using Arlequin ver. 3.0 (Excoffier et al. 2005) under the Tamura model with a gamma correction (α) = 0.167. Sequences were 572 bp in length with 528 bp having less than 5% missing data. One d-loop haplotype, Ns1D, was shared between 2 individuals within the La Bomba subpopulation (frequency = 0.061). The haplotype Ns2D was shared between 2 individuals within the Eastern Beach subpopulation (frequency = 0.105). Only 1 haplotype, Ns3D, was shared between the Eastern Beach and La Bomba subpopulations (1 Eastern Beach individual and 2 La Bomba individuals). All remaining individuals were represented by a unique haplotype (Ns4D to Ns30D relative frequencies = 0.030, La Bomba; Ns31D to Ns44D relative frequencies = 0.053, Eastern Beach).

Nucleotide composition for samples collected at La Bomba was 24.11% C, 8.62% G, 28.44% T, and 38.83% A. There were 485 usable loci with 99 polymorphic sites (*S*) with 23 transition sites, 23 transversion sites, and 63 indel sites. The haplotype diversity was 1.000 ± 0.0075 with $\pi = 0.019 \pm 0.009$. Nucleotide composition for samples collected at Eastern Beach was 24.39% C, 7.81% G, 28.25% T, and 39.55% A. There were 299 usable loci with 37 polymorphic sites with 18 transitions, 4 transversions, and 14 indel sites. Haplotype diversity was $h = 1.000 \pm 0.0185$ with $\pi = 0.019 \pm 0.011$.

Estimated Φ_{ST} values were higher for sequence data than microsatellites (Φ_{ST} = 0.165; p= 0.002). The subpopulation specific Φ_{ST} was 0.161 for La Bomba and 0.173 for

Eastern Beach. The overall $h = 1.000 \pm 0.0075$. The *Nm* between the subpopulations based on d-loop haplotypes = 2.524.

The sequence data was analyzed using TCS with a 95% connection limit and maximum 10 steps (Fig. 14). The resulting haplotype network included the same common haplotypes, Ns1D, Ns2D, and Ns3D. These haplotypes were returned at a higher frequency in TCS than in Arlequin. Haplotype Ns3D was present in 6 individuals shared between localities (2 from Eastern Beach, 4 from La Bomba). Haplotype Ns1D was also shared between localities (5 individuals— 2 from Eastern Beach, 3 from La Bomba). Haplotype Ns2D was found only within Eastern Beach (3 individuals). These individuals differed by missing or ambiguous characters. Ten haplotypes did not connect with the network under 95% connection limit or under lower connection limit of 90%. The haplotype network presented does not include these unconnected haplotypes (Fig. 13).

The parameters of population expansion based on a mismatch distribution were $\tau = 13.300$, $\theta_0 = 9.068$, and $\theta_1 = 50.664$. If k= 572 nucleotides, then $u = 4.576 \times 10^{-5}$, and using $t = \tau / 2u$, the time from the most recent population expansion was 145,323 yrs (generations).

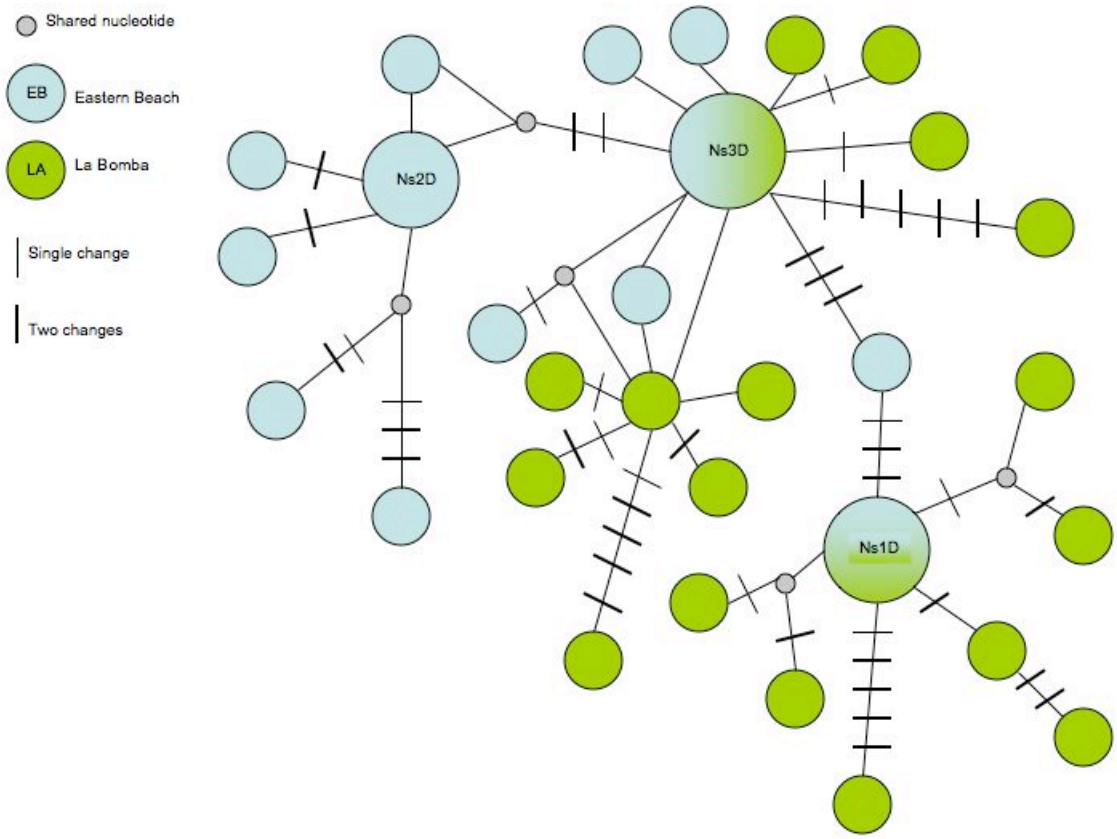

Figure 14. The TCS haplotype network for samples of *N. swarthi*. Lines connecting haplotypes (circles) represent a single step or nucleotide substitution. Thin hash lines indicate an additional single difference between connected haplotypes and thick hash lines indicate 2 differences. Haplotype Ns1D was present in 5 individuals, haplotype Ns2D was present in 3 individuals, and haplotype Ns3D was present in 6 individuals. Haplotypes Ns1D and Ns3D are shared between subpopulations.

NESORYZOMYS NARBOROUGHI

Microsatellite Results

Sixty-nine individuals of *N. narboroughi* comprised 3 sampling groups: Cabo Douglas (n= 55), Punta Espinoza (n= 9), and Crater Rim (n= 6). Of the 7 microsatellite loci successfully amplified, only 4 (Nect08, Nect12, Nect18, and Nect24) were polymorphic (P= 0.300). N_A for polymorphic loci ranged from 5 (Nect12) to 9 (Nect18) with a mean N_A = 7.5 alleles. The mean frequency of private alleles was 0.149. The allelic richness R_G per locus across all sample groups ranged from 2.562 to 4.370.

Linkage equilibrium tests were based on 600 permutations with an adjusted p-value = 0.00167. There was no evidence of linkage disequilibrium across loci (Table 8). Numerous microsatellite haplotypes were shared among samples from Cabo Douglas and between samples collected from Cabo Douglas and Punta Espinoza. *Nesoryzomys narboroughi* had a probability of identity comparable to *A. bauri*, but lower than *N. swarthi* (P_{ID} = 0.00361).

Table 8. Tests for linkage equilibrium p-values between loci in *N. narboroughi* (subpopulations and in the pooled population). Adjusted p-value = 0.00167.

Loci	Cabo Douglas	Crater Rim	Punta Espinoza	All *N. narboroughi*
Nect08 X Nect12	0.003	1.000	0.097	0.002
Nect08 X Nect18	0.640	1.000	0.703	0.665
Nect08 X Nect24	0.217	0.078	0.048	0.068
Nect12 X Nect18	0.588	0.780	0.883	0.647
Nect12 X Nect24	0.595	1.000	0.183	0.535
Nect18 X Nect24	0.213	1.000	1.000	0.262

The frequency of null alleles for most loci ranged from 0% to 29% (Table 9). Elevated estimated frequencies of null alleles in the Punta Espinoza and Crater Rim subpopulations may have been exacerbated by small samples sizes. The estimated null allele frequency for Nect24 was high for all subpopulations, which may be the result of allelic dropout.

Tests for recent bottleneck events varied depending on the model of mutation employed. Under the IAM and TPM models, there was no indication of a bottleneck (Cabo Douglas p= 0.104 and 0.195, respectively; Punta Espinoza p= 0.451 and 0.487, respectively; Crater Rim p= 0.128 and 0.108, respectively; pooled samples of *N. narboroughi* p= 0.463 and 0.191, respectively). Under the stricter and more appropriate SMM, there was some indication of a possible recent bottleneck for Cabo Douglas and across all samples (p= 0.028 and 0.027, respectively, but 0.348 and 0.458 for Crater Rim and Punta Espinoza, respectively). The allele frequency distribution for Crater Rim and Punta Espinoza was not as informative due to small sample size (Fig. 15).

Mutations per generation were θ_H = 0.985, 1.474, 1.095, and 1.185 for Cabo Douglas, Punta Espinoza, Crater Rim, and across pooled samples, respectively. The effective population size for each subpopulation was 2,463 (Cabo Douglas), 3,684 (Punta Espinoza), 2,737 (Crater Rim), and 2,961 (pooled *N. narboroughi*).

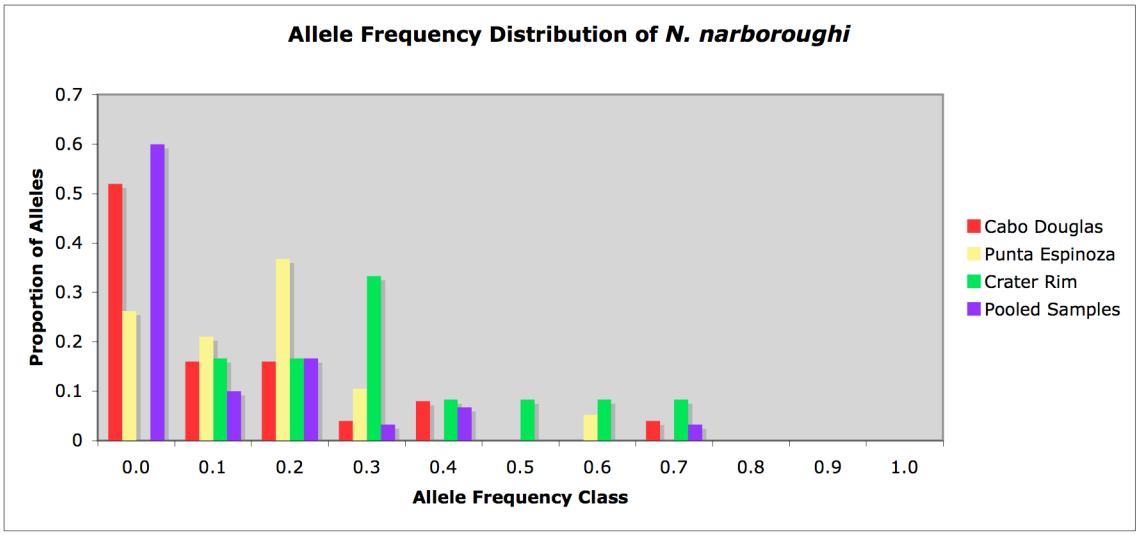

Figure 15. The allele frequency distribution of microsatellite loci in *N. narboroughi*

Tests for HWE indicated a deficiency of heterozygotes across 3 of 4 loci (Nect08 $p= 0.014$, Nect18 $p< 0.0001$, and Nect24 $p< 0.0001$) in samples collected from Cabo Douglas (Table 9). Tests for HWE indicated a deficiency of heterozygotes at only Nect12 in samples collected from Punta Espinoza ($p= 0.047$), while samples collected from Crater Rim showed deviation from HWE at 2 loci (Nect18 $p= 0.004$ and Nect24 $p= 0.021$; Table 9).

Fixation indices were calculated across all loci. All indices, θ_{WC}, G'_{ST}, G''_{ST}, and R_{ST}, indicated moderate differentiation between subpopulations (0.071, 0.053, 0.123, and 0.059 respectively; Table 10). Slatkin's R_{ST} across all loci values were 0.059 (weighted), 0.058 (Goodman's adjusted), and 0.056 (unweighted).

Table 9. Allelic diversity and deviation from Hardy-Weinberg proportion among microsatellite loci in *N. narboroughi*. Number of alleles N_A, allelic richness R_G, gene diversity H_S, observed and expected heterozygosity (H_O and H_E), *p*-values for Hardy-Weinberg Equilibrium, and F_{IS} per locus per subpopulations are included. Numbers in parentheses represent sample size.

Locus	Number of Alleles N_a	Allelic Richness R_G	Gene Diversity H_S	Freq of Null Alleles r	Observed Heter. H_O	Expected Heter. H_E	HWE *p*-value	Theta-H θ_H	Fixation Index F_{IS}
Cabo Douglas (55)									
Nect08 (51)	7	3.928	0.695	0.104	0.608	0.694	0.014	2.268	0.125
Nect12 (55)	3	1.995	0.338	0.058	0.273	0.337	0.368	0.509	0.193
Nect18 (55)	8	4.341	0.775	0.031	0.727	0.775	<0.0001	3.444	0.062
Nect24 (43)	7	3.723	0.680	0.277	0.256	0.675	<0.0001	2.079	0.624
Overall:	= 25	3.497	0.395	0.117	0.466	0.620	<0.0001	1.660	0.251
Punta Espinoza (9)									
Nect08 (9)	6	4.851	0.806	0.000	0.889	0.810	0.306	4.276	-0.103
Nect12 (9)	4	3.941	0.792	0.208	0.778	0.791	0.047	3.781	0.018
Nect18 (9)	4	3.105	0.569	0.000	0.667	0.575	0.596	1.354	-0.171
Nect24 (7)	5	4.581	0.833	0.204	0.429	0.802	0.057	4.056	0.486
Overall:	= 19	4.120	0.530	0.103	0.690	0.745	0.050	2.693	0.079
Crater Rim (6)									
Nect08 (5)	3	3.000	0.700	0.016	0.600	0.689	0.620	2.214	0.143
Nect12 (6)	2	2.000	0.500	0.172	0.167	0.409	0.273	0.692	0.615
Nect18 (6)	4	3.818	0.650	0.205	0.167	0.712	0.004	2.474	0.783
Nect24 (6)	4	3.985	0.850	0.294	0.333	0.803	0.021	4.077	0.608
Overall:	= 13	3.201	0.478	0.170	0.317	0.653	0.005	1.891	0.539

Table 10. Genetic diversity indices per locus for the pooled population of *N. narboroughi*. Nei's within sample gene diversity or subpopulation heterozygosity (H_S), Nei's overall gene diversity independent of sample size H'_T , Weir and Cockerham's F (F_{IT}), θ_{WC} (F_{ST}), f (F_{IS}), Nei's gene divergence among samples independent of sample size (D'_{ST}), Nei's measure of heterozygosity deficit among populations (G_{ST}) and independent of sample size (G'_{ST}), Hedrick's standardized G_{ST} measure (G''_{ST}), and Slatkin's weighted R_{ST} are included.

Locus	H_S	H'_T	F	θ_{WC}	f	D_{ST}	D'_{ST}	G_{IS}	G_{ST}	G'_{ST}	G''_{ST}	R_{ST}
Nect08	0.733	0.740	0.107	0.018	0.091	0.005	0.007	0.047	0.006	0.009	0.039	0.043
Nect12	0.516	0.630	0.373	0.234	0.181	0.076	0.113	0.214	0.128	0.180	0.401	0.080
Nect18	0.698	0.766	0.188	0.097	0.101	0.045	0.068	0.255	0.061	0.089	0.343	0.126
Nect24	0.784	0.750	0.588	-0.030	0.600	-0.023	-0.034	0.567	-0.030	-0.045	-0.248	-0.025
Overall	***0.546***	***0.577***	***0.302***	***0.071***	***0.249***	***0.021***	***0.031***	***0.281***	***0.036***	***0.053***	***0.123***	***0.059***

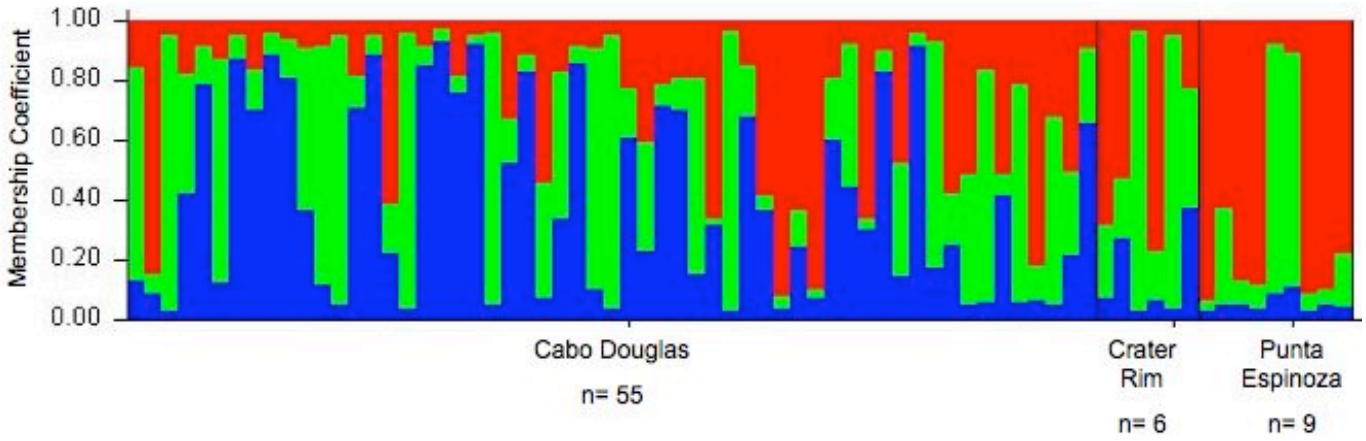

Figure 16. *Structure* Q-plot showing the level of genetic substructure in *N. narboroughi* (K= 3)

Microsatellite data indicated population substructure, though this may be due to small sample size (Fig. 16). The number of migrants after a correction for size was *Nm*= 0.3199.

D-loop Results

Fifty-seven individuals were sequenced, comprising 3 sampling groups: Cabo Douglas (n= 50), Punta Espinoza (n= 4), and Crater Rim (n= 3). Sequences were 669 bp in length and 621 bp had less than 5% missing data. The Tamura model was used with gamma correction α= 0.226. The Cabo Douglas subpopulation had 1 common haplotype, Nn1D, represented by 3 individuals (frequency = 0.060). No haplotypes were shared between subpopulations. All other individuals were represented by a unique haplotype (Nn2D to Nn47D relative frequencies = 0.020, Cabo Douglas; Nn48D to Nn51D relative frequencies = 0.25, Punta Espinoza; Nn52D to Nn54D relative frequencies = 0.333, Crater Rim).

In the Cabo Douglas subpopulation, sequences were 620 bp in length with less than 5% missing data and 185 polymorphic sites (*S*). The nucleotide composition was 25.41% C, 10.42% G, 30.78% T, and 33.39% A with 30 transition sites, 40 transversions, and 123 indel sites. Haplotype diversity was high with low nucleotide diversity (*h*= 1.000 ± 0.0040; π= 0.009 ± 0.005). In the Punta Espinoza subpopulation, sequences contained 532 usable loci with only 31 polymorphic sites (*S*). Nucleotide composition was 24.90% C, 10.95% G, 30.85% T, and 33.30% A with 5 transitions, 4 transversions, and 22 indel sites. Haplotype diversity was 1.000 ± 0.177 (π= 0.0089 ± 0.0066).

Sequences from samples collected from the Crater Rim subpopulation contained 572 usable loci with 23 polymorphic sites. Nucleotide composition was 25.36%C, 10.51% G, 30.58% T, and 33.55% A with 5 transitions, 6 transversions, and 13 indel sites. Haplotype diversity was 1.000 ± 0.2722 (π= 0.0126 ± 0.0101).

An AMOVA analysis generated an Φ_{ST} = 0.009 (p= 0.413) indicating little differentiation between subpopulations. Pairwise Φ_{ST} values were: Cabo Douglas-Punta Espinoza, Φ_{ST} = 0.042 (p= 0.189± 0.038); Cabo Douglas-Crater Rim, Φ_{ST} = -0.033 (p= 0.649 ± 0.038); Punta Espinoza- Crater Rim, Φ_{ST} = -0.049 (p= 0.595 ± 0.028). The number of migrants corresponding to the Crater Rim subpopulation approached infinity. The *Nm* between Cabo Douglas and Punta Espinoza = 5.742.

A haplotype network under a 95% connection limit with 11 steps was constructed using TCS (Fig. 17). Four haplotypes were shared among individuals. The haplotype Nn1D was present in 6 individuals from Cabo Douglas. Two additional haplotypes were present in 2 individuals and a third haplotype was present in 4 individuals, all of which were from Cabo Douglas. These haplotypes differed by missing or ambiguous characters. There were no haplotypes shared between subpopulations. Nine haplotypes did not connect to the network under 95% and lower 90% connection limit.

The parameters of population expansion based on a mismatch distribution were τ= 4.000, θ_0 = 14.180, and θ_1 = 65.861. If k= 669 nucleotides, then u= 5.325 x 10^{-5}, and using t= τ/ 2u, the time from the most recent population expansion was 37, 369 yrs (generations).

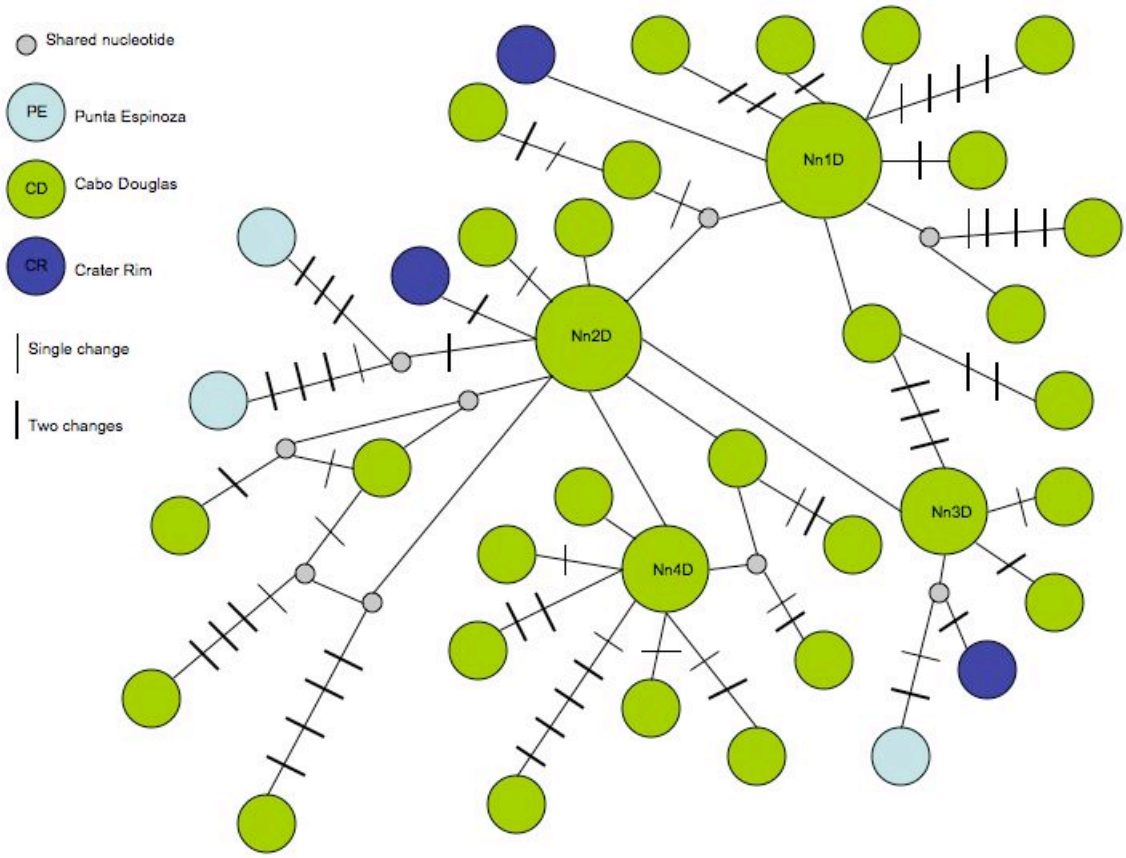

Figure 17. The TCS haplotype network for *N. narboroughi*. Lines connecting haplotypes (circles) represent a single step or nucleotide substitution. Thin hash lines indicate an additional single difference between connected haplotypes and thick hash lines indicate 2 differences. Haplotype Nn1D is present in 6 individuals. Haplotype Nn2D is present in 4 individuals. Haplotypes Nn3D and Nn4D are both represented by 2 individuals each.

NESORYZOMYS FERNANDINAE

Microsatellite Results

Seven loci successfully amplified in 38 samples of *N. fernandinae*, of which 5 loci (Nect08, Nect12, Nect14, Nect18, and Nect24) were polymorphic (P= 0.417). Number of alleles ranged from 2 (Nect14) to 11 (Nect24) with mean N_A= 7.2. Allelic richness ranged from R_G= 2.000 to 8.576. Mean frequency of private alleles = 0.097.

Samples were collected from 2 locations, Cabo Douglas (n= 16) and Crater Rim (n= 21). Only 1 microsatellite genotype across 5 loci was shared between the locations. All other individuals represented unique haplotypes. The probability of identity for *N. fernandinae* was 0.00039.

All loci were tested for linkage equilibrium and after 400 permutations (adjusted p-value = 0.0025), there was no evidence of linkage disequilibrium (Table 11).

Table 11. Tests for linkage equilibrium *p*-values between loci in *N. fernandinae* in each subpopulation and in the pooled population. Adjusted *p*-value= 0.0025.

Loci	Cabo Douglas	Crater Rim	Pooled *N. fernandinae*
Nect08 X Nect12	0.845	0.083	0.253
Nect08 X Nect14	0.758	0.625	0.673
Nect08 X Nect18	0.885	0.288	0.500
Nect08 X Nect24	1.000	1.000	1.000
Nect12 X Nect14	0.468	0.958	0.763
Nect12 X Nect18	0.848	0.198	0.385
Nect12 X Nect24	1.000	1.000	1.000
Nect14 X Nect18	0.200	0.105	0.053
Nect14 X Nect24	1.000	0.380	0.463
Nect18 X Nect24	1.000	0.288	0.315

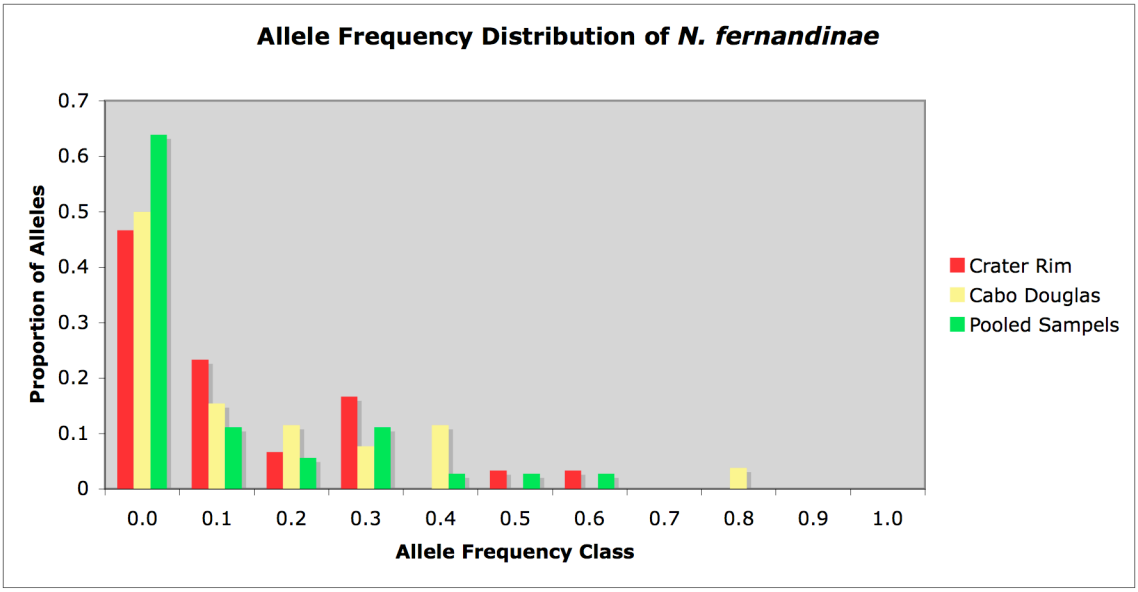

Figure 18. The allele frequency distribution of microsatellite loci in *N. fernandinae*

Subpopulations and the pooled population demonstrated no evidence of a recent bottleneck under any mutation model (Cabo Douglas: IAM p= 0.055, TPM p= 0.647, SSM p= 0.112; Crater Rim: IAM p= 0.051, TPM p= 0.057, SSM p= 0.102; pooled *N. fernandinae*: IAM p= 0.054, TPM p= 0.361, SSM p= 0.118). The allele frequency distribution supported the sign test results (Fig. 18). The Cabo Douglas subpopulation had null allele frequencies ranging from ~10% to 20%. The high frequency of null alleles across all loci is likely the result of genetic drift. The Crater Rim subpopulation exhibited a high null allele frequency for Nect24 (Table 12).

The Crater Rim subpopulation had a greater θ_H than the Cabo Douglas subpopulation (2.347 and 1.852, respectively). The N_e for each subpopulation and the

pooled population was N_e= 5,868 (Crater Rim) N_e = 4,629 (Cabo Douglas), and N_e= 5,242 (pooled *N. fernandinae*). The *Nm* between subpopulations corrected for size = 1.206.

Tests for HWE indicated a heterozygote deficiency at 3 loci in samples collected at Cabo Douglas (Nect12 p= 0.005, Nect18 p= 0.021, and Nect24 p= 0.001). In contrast, tests for HWE indicated a heterozygote deficiency at only 1 locus (Nect24) in samples collected at Crater Rim ($p < 0.0001$; Table 12).

Genetic differentiation indices were calculated. Only 1 locus (Nect12) exhibited an excess of heterozygotes in pooled samples (f = -0.025). The θ_{WC} overall loci was low indicating little to no population differentiation (θ_{WC} = 0.013). Estimated Nei's G'_{ST} (0.013) and Hedrick's G''_{ST} (0.032) also indicated the majority of variation was within subpopulations, not between Cabo Douglas and Crater Rim. Slatkin's R_{ST}, however, showed slightly more differentiation than the other estimates (0.094 (weighted), 0.058 (Goodman's adjusted), and 0.052 (unweighted)). Interestingly, this is the highest R_{ST} reported in this study. Results from *structure* 2.2 (Falush et al. 2007; Pritchard et al. 2000) did not detect any population substructure (Fig. 19).

Table 12. Allelic diversity and deviation from Hardy-Weinberg proportion among microsatellite loci in *N. fernandinae*. Number of alleles N_A, allelic richness R_G, gene diversity H_S, observed and expected heterozygosity (H_O and H_E), *p*-values for Hardy-Weinberg Equilibrium, and F_{IS} per locus per subpopulations are included. Numbers in parentheses represent sample size.

Locus	Number of Alleles N_a	Allelic Richness R_G	Gene Diversity H_S	Freq of Null Alleles r	Observed Heter. H_O	Expected Heter. H_E	*p*-value	Theta-H θ_H	Fixation Index F_{IS}
Cabo Douglas (16)									
Nect08 (15)	6	5.857	0.798	0.098	0.800	0.798	0.080	3.943	-0.003
Nect12 (16)	6	5.615	0.746	0.105	0.625	0.742	0.005	2.875	0.162
Nect14 (11)	2	2.000	0.318	0.099	0.182	0.312	0.279	0.453	0.429
Nect18 (14)	4	3.571	0.599	0.202	0.286	0.587	0.021	1.423	0.523
Nect24 (12)	8	7.663	0.814	0.118	0.667	0.808	0.001	4.208	0.181
Overall:	= 26	4.941	0.439	0.165	0.512	0.649	0.005	1.852	0.219
Crater Rim (21)									
Nect08 (20)	9	7.578	0.822	0.046	0.762	0.812	0.438	4.315	0.088
Nect12 (21)	7	6.157	0.780	0.000	0.909	0.793	0.892	3.827	-0.160
Nect14 (17)	2	2.000	0.471	0.030	0.444	0.489	1.000	0.957	0.000
Nect18 (21)	4	3.559	0.588	0.000	0.682	0.601	0.930	1.509	-0.134
Nect24 (14)	8	7.687	0.860	0.334	0.200	0.809	<0.0001	4.241	0.751
Overall:	= 30	5.396	0.566	0.144	0.599	0.701	<0.0001	2.343	0.146

Table 13. Genetic diversity indices per locus for the pooled population of *N. fernandinae*. Nei's within sample gene diversity or subpopulation heterozygosity (H_S), Nei's overall gene diversity independent of sample size H'_T, Weir and Cockerham's F (F_{IT}), θ_{WC} (F_{ST}), f (F_{IS}), Nei's gene divergence among samples independent of sample size (D'_{ST}), Nei's measure of heterozygosity deficit among populations (G_{ST}) and independent of sample size (G'_{ST}), Hedrick's standardized G_{ST} measure (G''_{ST}), and Slatkin's weighted R_{ST} are included.

Locus	H_S	H'_T	F	θ_{WC}	f	D_{ST}	D'_{ST}	G_{IS}	G_{ST}	G'_{ST}	G''_{ST}	R_{ST}
Nect08	0.810	0.743	0.043	-0.008	0.050	-0.003	-0.105	0.043	-0.004	-0.008	-0.038	-0.006
Nect12	0.762	0.831	0.046	0.069	-0.025	0.028	0.029	-0.003	0.035	0.069	0.259	-0.029
Nect14	0.394	0.624	0.155	0.028	0.131	0.006	0.284	0.173	0.015	0.029	0.035	0.028
Nect18	0.592	0.681	0.111	-0.022	0.130	-0.007	0.001	0.196	-0.012	-0.024	-0.047	0.016
Nect24	0.838	0.700	0.493	-0.007	0.496	-0.002	-0.064	0.474	-0.003	-0.006	-0.034	0.250
Overall	***0.679***	***0.716***	***0.177***	***0.013***	***0.166***	***0.004***	***0.029***	***0.181***	***0.006***	***0.013***	***0.032***	***0.094***

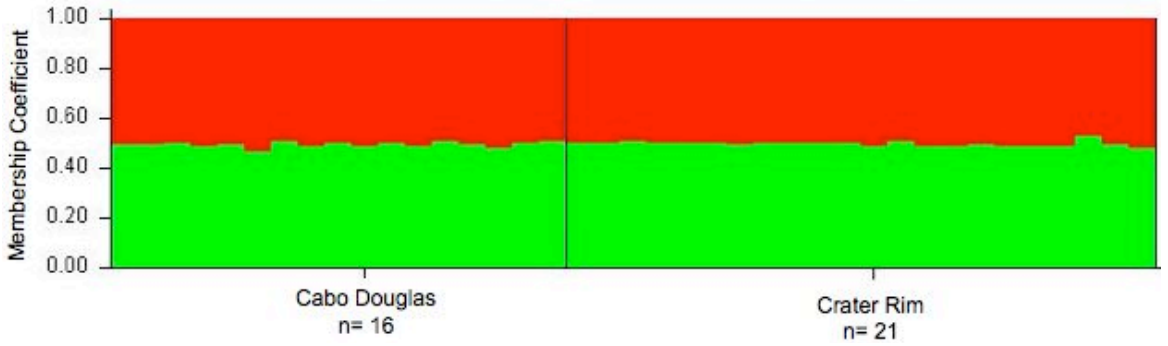

Figure 19. *Structure* Q-plot showing the level of genetic substructure in *N. fernandinae* (K= 2)

D-loop Results

Sequences from 27 individuals were obtained from 2 subpopulations (Cabo Douglas n=14 and Crater Rim n=13, respectively). The Tamura model with a gamma correction α= 0.3704 was employed. Unique haplotypes were documented in both subpopulations (Nf1D to Nf14D relative frequencies = 0.0714, Cabo Douglas; Nf15D to Nf27D relative frequencies = 0.0769, Crater Rim). No haplotypes were shared between subpopulations.

Sequences obtained from the Cabo Douglas subpopulation were 563 bp in length with less than 5% missing data and 79 polymorphic sites (*S*). Haplotype diversity was high with low nucleotide diversity (h= 1.000 ± 0.0270; π = 0.0167 ± 0.0092). Nucleotide composition was 26.09% C, 8.47% G, 27.57% T, and 37.87% A with 21 observed transitions, 22 transversions, and 37 indels. Sequences obtained from the Crater Rim subpopulation were 527 bp in length with less than 5% missing data and 111 polymorphic sites (*S*). Haplotype diversity was high (h= 1.000 ± 0.0302; π = 0.0272 ± 0.0147). Nucleotide composition was 26.01% C, 8.72% G, 27.45% T, and 37.82% A with 40 transitions, 30 transversions, and 44 indels. The Φ_{ST} value generated from an AMOVA analysis was low and not significant (0.0283; p= 0.074 ± 0.0083). The subpopulation specific Φ_{ST} for Cabo Douglas was 0.044, whereas the subpopulation specific Φ_{ST} for Crater Rim was 0.011. The number of migrants between the two subpopulations was larger than with microsatellites, Nm= 17.157.

A haplotype network of *N. fernandinae* samples under 95% connection limit with a maximum 10 steps was generated (Fig. 20). No shared haplotypes were found between

Cabo Douglas and Crater Rim subpopulations. Eight haplotypes, 3 from Cabo Douglas and 5 from Crater Rim, did not connect to the haplotype network and failed to connect with each other under 90% connection limit.

The parameters of population expansion based on a mismatch distribution were $\tau = 11.578$, $\theta_0 = 10.294$, and $\theta_1 = 151.406$. If k= 621 nucleotides, then u= 4.987 x 10^{-5}, and using t= τ/ 2u, the time from the most recent population expansion was 116,526 yrs (generations).

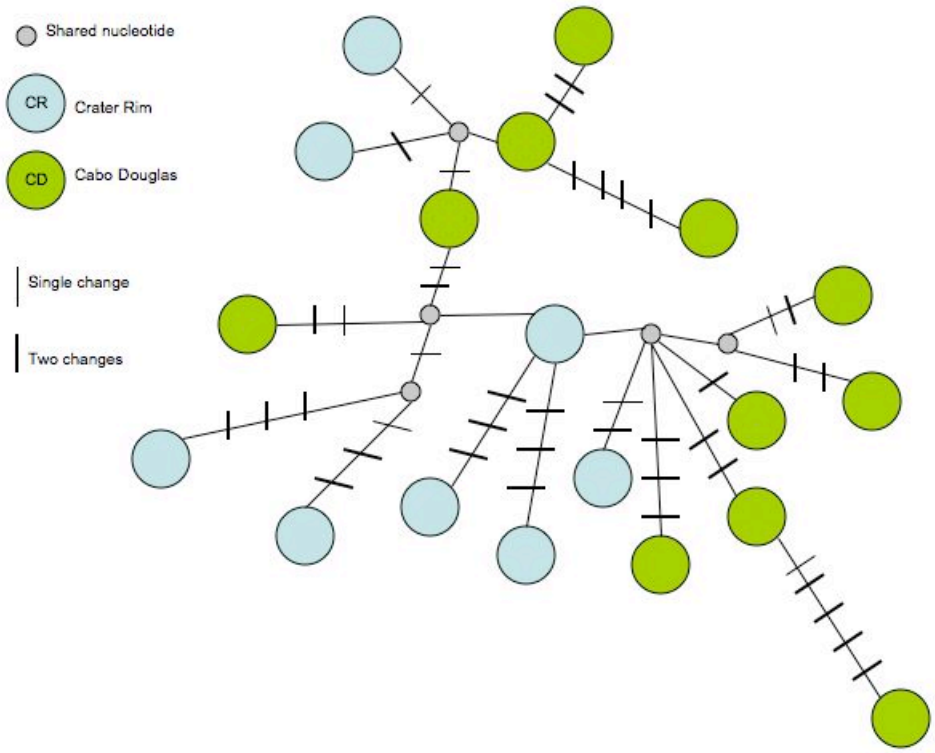

Figure 20. The TCS haplotype network for *N. fernandinae*. Lines connecting haplotypes (circles) represent a single step or nucleotide substitution. Thin hash lines indicate an additional single difference between connected haplotypes and thick hash lines indicate 2 differences. There were no haplotypes shared within or between subpopulations.

DISCUSSION

Quantifying and understanding the genetic structure of natural populations is a hallmark objective of evolutionary biology and a management necessity for conservation. Further, island ecosystems have allowed scientists a laboratory to study evolutionary theory with clarity and precision, a way that cannot be duplicated easily in the contiguous ecosystems of the mainland. Island populations are expected to suffer high levels of inbreeding due to bottlenecks and subsequent small population sizes (Frankham 1998). Although all 4 endemic rodent species in the Galápagos Islands exhibited high d-loop haplotype diversity, they exhibited varying levels of heterozygosity and population structure (Table 14). Similar patterns of high haplotype diversity and low genetic structuring have been recorded in other studies (Appendix– Table 16).

Table 14. Diversity indices for *Nesoryzomys* spp. and *A. bauri* generated using microsatellites and d-loop data.

			Microsatellite				D-loop	
Species	θ_{WC}	D'_{ST}	R_{ST}	G_{ST}	G'_{ST}	G''_{ST}	Φ_{ST}	***t* (yrs)**
O. bauri	0.158	0.088	0.034	0.083	0.153	0.241	0.007	45,568
N. swarthi	-0.012	-0.009	-0.019	-0.006	-0.012	-0.038	0.165	145,323
N. narboroughi	0.071	0.031	0.059	0.036	0.053	0.123	0.009	37,369
N. fernandinae	0.013	0.009	0.094	0.006	0.013	0.032	0.028	116,526

AEGIALOMYS BAURI

Aegialomys bauri was polymorphic for 5 microsatellite loci and exhibited no signs of linkage disequilibrium between loci (Table 2). Although the number of alleles and allelic richness per locus were moderate (Table 3), the probability of identity was high (P_{ID}= 0.00385 or 1 in 260). The overall observed heterozygosity for *A. bauri* was lower than expected (H_O= 0.423 and H_E= 0.560, respectively). A deficiency of heterozygosity for all microsatellite loci and an overall F_{IS} of 0.388 was detected in Sampling Period 1 in 1997-1999 (Table 4). All loci in this subpopulation had a high null allele frequency (~10% to 38%). Given the high F_{IS} values and significant *p*-values for HWE tests for all loci (Tables 3 and 4), the high null allele frequencies are likely the result of genetic drift and not mistyping errors. Individuals collected during 2006 (Sampling Period 2), however, exhibited heterozygote excess in 3 of 4 loci with an overall F_{IS}= -0.047. Only locus Nect24 exhibited a deficit (p= 0.016). All loci exhibited a null allele frequency of 0%.

The effective population size estimated using data from microsatellites was higher in Sampling Period 1 than in Sampling Period 2 (N_e= 3,517 and 1,290, respectively). Clark (1980), estimated the population size of *A. bauri* to be 10,000 < N < 100,000 based on trapline success. Taken at face value, these estimates seem to show a pattern of decline, possibly due to inbreeding and high levels of local extinctions, although neither the sign-test nor the allele frequency distribution indicated a recent population bottleneck. There may be too few loci for an accurate sign-test, however, and the bottleneck may be

too recent to detect with a mode-shift distribution test. Additionally, Clark (1980) employed a more rigorous sampling regime and his estimates were not calculated using microsatellite data, making direct comparisons of population estimates difficult.

At the microsatellite level, *A. bauri* exhibited the highest *F*-statistics in the study. Weir and Cockerham's θ_{WC}, Nei's G'_{ST} , and Hedrick's G''_{ST} indicated great differentiation (0.158, 0.153, and 0.241 respectively), however, Slatkin's weighted R_{ST} indicated little differentiation (0.034).

The trap lines of Sampling Period 1 and Sampling Period 2 radiated from the beach inland and there were no physical barriers between trapping localities that would have inhibited dispersal or gene flow (Edwards, pers. comm.). Though not geographically separate, the subpopulations are temporally separate by nearly 9 years. However, such a strong genetic differentiation would require a catastrophic event to have occurred between sampling periods.

The winter of 1997-1998 was characterized by a severe El Niño Southern Oscillation Event (ENSO) and the winter of 2002-2003 El Niño event was comparatively milder but still with warmer than normal sea temperatures (Fig. 21). Sampling Period 2, collected following both ENSO events, was in HWE, whereas Sampling Period 1, collected prior to El Niño, was not. *Aegialomys bauri*'s life history and litter size are closely tied to the seasonal rains and resulting food availability (Clark 1980). A similar vegetation boom that altered the landscape of Isla Isabela following the El Niño in 1980 could have occurred on Isla Santa Fe. Since fewer samples were collected with a similar

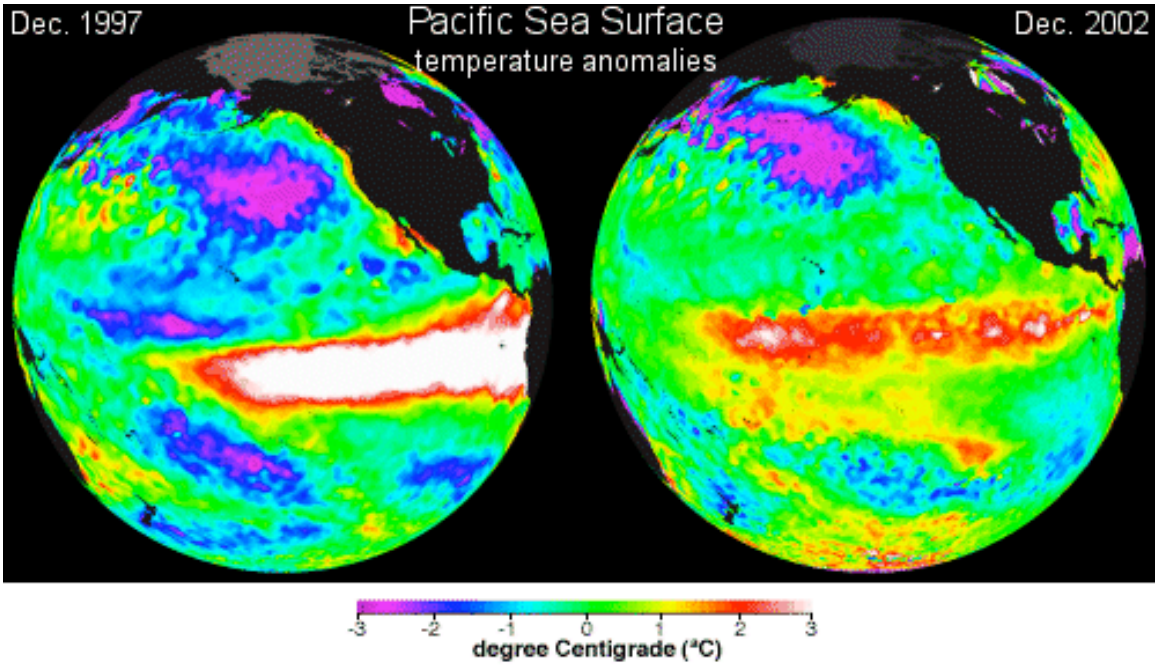

Figure 21. A comparison of El Niño's warm strip in Dec. 1997 and Dec. 2002. Sea surface temperature anomalies in these maps were computed from measurements of sea surface temperature collected by the AVHRR sensor on the NOAA polar orbiting satellites. Taken from http://science.nasa.gov

trapping effort in 2006 compared to 1997 (Edwards, pers. comm.), the population of *A. bauri* on Isla Santa Fe appears to have declined in the years following ENSO. If the level of genetic divergence at the microsatellite level is the result of a cyclic population turnover following ENSO, a similar effect is not expected been seen at the mitochondrial level due to the time scale of the event and mutation rate of the d-loop. Indeed, the Φ_{ST} estimate was low (0.007) and did not detect population substructure between sampling periods.

Aegialomys bauri exhibited high haplotype diversity and low nucleotide diversity (h= 1.000 ± 0.0034 and π= 0.009 ± 0.005, respectively). These values suggest closely related haplotypes and are depicted in the web-like haplotype network (Fig. 11). Patton

and Hafner (1983) estimated the arrival of *A. bauri* to the Galápagos Archipelago to have occurred as early as a few hundred years ago. The high number of haplotypes, however, is not congruent with that estimate. The estimated time since the most recent population expansion (45,568 yrs) predates Patton and Hafner's estimate. If Patton and Hafner's estimated time of arrival is accurate, however, then *A. bauri* would have to be the result of numerous colonization events with large founding population sizes.

As of 2006, there were no introduced rodents or cats on the Isla Santa Fe (Edwards, pers. comm.). Given the small area of Isla Santa Fe (24 km^2) and low trap success of recent expeditions, this species is still highly susceptible to extinction. A captive breeding program would aid in maintaining the current levels of genetic diversity and could be used as a "safety net" in the event that observed population declines are on going.

NESORYZOMYS SWARTHI

The 5 microsatellite loci studied were in linkage equilibrium both at the subpopulation and specific level. Microsatellite loci demonstrated moderate to high levels of allelic richness and gene diversity. The probability of identity estimated for *N. swarthi* was lower than the value estimated for *A. bauri* (P_{ID}= 0.00018 or 1 in 5,555).

Nect14 was represented by 7 alleles in 9 individuals. Although amplification was inconsistent, this locus could be highly informative in diversity or parentage analyses. Future work should include modification of the Nect14 primer and protocol until reliable

amplification is attained. Since null allele frequencies are high across all loci, we cannot distinguish between the presence of null alleles versus genetic drift (Table 6). Genetic drift is expected to have the same effect on all loci in the genome of a small population (Hedrick 2005b). Therefore, the discrepancy between expected and observed heterozygosity may be genuine and not an artifact of scoring error.

Most loci from the La Bomba and Eastern Beach samples were not in HWE. Interestingly, both subpopulations showed an excess of heterozygosity at Nect12. Both the La Bomba and Eastern Beach subpopulations contained unique alleles (Appendix—Table 19). The mean frequency of private alleles was low (0.037). The *Nm* between La Bomba and Eastern Beach = 3.472. All parameters of genetic differentiation between the two subpopulations across all loci were low (θ_{WC}= -0.012, G'_{ST}= -0.012, G''_{ST}= -0.038, and R_{ST}= -0.019). While some variation between the 2 subpopulations was documented, very little was estimated from microsatellite data.

Nesoryzomys swarthi had the lowest *F*- statistic microsatellite values reported in this study. Given the close proximity of the 2 localities of *N. swarthi* and the extremely limited available habitat, this is most likely a single panmictic population on Isla Santiago.

Contrary to the hypothesis, d-loop haplotype diversity for the pooled population was very high with low nucleotide diversity (h= 1.000 ± 0.0075 and π= 0.019). There were 3 common haplotypes, 2 of which were shared between La Bomba and Eastern Beach (Fig. 14). Φ_{ST} values generated using data from mtDNA were higher than those estimated using microsatellites (0.165, p= 0.002). The appearance of population

substructure at the mitochondrial level is most likely an artifact of female philopatry and smaller home ranges (Harris and McDonald 2007; Harris et al. 2006). The estimated number of migrants was slighter lower than the *Nm* generated using microsatellite data (2.524 versus 3.472, respectively). *Nesoryzomys swarthi* had the longest time since population expansion (145,323 yrs) based on d-loop data.

The effective population size for *N. swarthi* was 5,056. This estimate seems high given *N. swarthi* was, until recently, thought to be extinct (Dowler et al. 2000). Larger N_e estimates typically indicate low differentiation across the population and a lack of recent expansion—the latter being the most pertinent and prophetic as *N. swarthi* is currently restricted to a small area of the island. Despite the fact that suitable habitat is fragmented and isolated, no evidence of a recent bottleneck was detected under any model of mutation.

Given its remaining small and restricted population, *Nesoryzomys swarthi* is listed currently as "Vulnerable" (Tirira et al. 2008b). The precarious situation is further exacerbated by the presence of the invasive *Rattus rattus* and *Mus musculus* on Isla Santiago. When ecologically similar species are brought together without the co-evolutionary time needed to create niche partitioning (as occurs with invasive species) the struggle for coexistence can intensify. Current evidence indicates populations of *Nesoryzomys swarthi* and *Rattus rattus* have been sympatric for almost 400 years. *Rattus rattus* populations crash during periods of drought when foods are limited while *N. swarthi* maintain a more stable population year round (Harris and MacDonald 2007). This regular disequilibrium may afford *N. swarthi* a temporary reprieve from competition

with *Rattus rattus*. Further, *Opuntia galapageia* density is highest on Isla Santiago at La Bomba. The fluctuating severity of competition pressure in conjunction with a selected diet of *Opuntia galapageia* has allowed the single remaining population of *N. swarthi* to persist, however tentatively, at La Bomba. These findings highlight the importance of native vegetation in the Galápagos. Conservation programs involving *N. swarthi* should include subsequent protection of *Opuntia galapageia*. It would not be unreasonable to hypothesize that extirpation of this cactus species could lead to further decline, or extinction, of the remaining *N. swarthi* on Isla Santiago.

NESORYZOMYS NARBOROUGHI

Nesoryzomys narboroughi was polymorphic for 4 loci and was the only species included in this study that was monomorphic for Nect14. All loci were in linkage equilibrium. *Nesoryzomys narboroughi* showed slightly less allelic diversity than other species in this study. Consequently, the probability of identity was high (P_{ID}= 0.00361 or 1 in 277). As in *N. swarthi*, the locus Nect24 exhibited a high frequency of null alleles (24%).

Samples collected from Cabo Douglas were not in HWE and exhibited a heterozygote deficit at 3 of 4 loci (Table 5). Samples collected from Punta Espinoza were in HWE at all but 1 locus (Nect12 p= 0.047). However, sample sizes from Punta Espinoza and Crater Rim were small (n=9 and 6, respectively). As hypothesized, all estimates of population differentiation indicated moderate differentiation (θ_{WC}= 0.071,

G'_{ST}= 0.053, G''_{ST}= 0.123, and R_{ST}= 0.059). The number of migrants was low (*Nm*= 0.312), however. The effective population size for pooled *N. narboroughi* was 2,961, the lowest N_e value estimated in the study.

Nesoryzomys narboroughi, formally listed as "Near Threatened" in 2002, was listed recently as "Vulnerable" in 2008 due to declining population trends (Tirira et al. 2008b). Overall, the population of *N. narboroughi* seems to have undergone a recent bottleneck (under the SMM). Populations generally develop heterozygosity excess at selectively neutral loci after a bottleneck (Cornuet and Luikart 1996), and an excess was reported at 2 loci in the Punta Espinoza subpopulation (Nect08 and Nect18; Table 9).

Bottlenecks can increase demographic stochasticity, inbreeding, loss of genetic variation, and fixation of mildly deleterious alleles. These effects can reduce evolutionary potential and increase the probability of population extinction (Cornuet and Luikart 1996; Luikart et al. 1998). It is especially important to identify recent bottlenecks (i.e. bottlenecks within the past few dozen generations), because these populations are at a higher risk of extinction. It is important to note that, although they likely have lost rare alleles, populations that have undergone a recent bottleneck may still retain substantial heterozygosity and genetic variation. Both of these values are lost more slowly than allelic variation and have a greater impact on fitness (Cornuet and Luikart 1996; Luikart et al. 1998). Therefore, if recently bottlenecked populations are identified, biologists may be able to minimize the loss of heterozygosity and genetic variation in the population.

All subpopulations of *N. narboroughi* exhibited high haplotype diversity with low nucleotide diversity. Variance for Punta Espinoza and Crater Rim was high, most likely

due to the small sample size. The generated haplotype network showed slight interconnectivity between haplotypes, but no haplotypes were shared between subpopulations (Fig. 17). This may have been an artifact of small sample size, and further sampling is recommended. The *Nm* between Cabo Douglas and Punta Espinoza subpopulations was 5.742, whereas the *Nm* corresponding to Crater Rim approached infinity. Overall Φ_{ST} for mtDNA was low and not significant (0.009; p= 0.413) indicating little to no differentiation between subpopulations. Interestingly, *N. narboroughi* had the most recent time since population expansion (35,369 yrs), suggesting some severe bottleneck may have happened at that time.

Moderate differentiation was indicated by microsatellite data, suggesting *N. narboroughi* may consist of distinct subpopulations. Any *ex situ* breeding programs should include individuals from all localities. There is a complete lack of ecological studies on the rodents of Isla Fernandina. Potential competition pressure and niche partitioning between sympatric populations of *N. narboroughi* and *N. fernandinae* has not been studied. Ecological interactions between these 2 species should be examined in an effort to identify potential population fluctuations caused by such competitive interactions.

NESORYZOMYS FERNANDINAE

No signs of linkage disequilibrium were indicated among the 5 loci amplified for *N. fernandinae* samples (Table 11). All loci exhibited allelic richness values comparable

to those estimated from the other species of *Nesoryzomys* included in this study. The probability of identity estimated (P_{ID}= 0.00039 or 1 in 2,564) was lower than the value estimated from sympatric *N. narboroughi*, but higher than the value estimated from *N. swarthi*. The Cabo Douglas subpopulation exhibited deviations from Hardy-Weinberg at 3 of 5 loci (Nect12, Nect18, Nect 24), whereas the Crater Rim subpopulation exhibited a deficit of heterozygosity at a single locus (Nect24; Table 12). As seen in the other endemic species, Nect24 had a high null allele frequency (22%). The Crater Rim subpopulation exhibited an excess of heterozygotes at 2 loci (Nect12 and Nect18). The Crater Rim subpopulation appears to be genetically stable given the aforementioned data.

The effective population sizes were: Cabo Douglas (5,868), Crater Rim (4,629), and pooled samples (5,242). These were the highest N_e values reported in this study. These estimates likely reflect the lack of population expansion on the island. Despite sparse suitable habitat on Isla Fernandina, there was no apparent evidence of a recent population bottleneck at either subpopulation. This does not mean that *N. fernandinae* is immune to population fluctuations or the risk of extirpation.

The level of genetic differentiation between subpopulations varied between estimates (Table 13). Both θ_{WC} and G'_{ST} were 0.013, indicating low differentiation. Slatkin's R_{ST}, considered a more accurate measure for microsatellite data, exhibited moderate genetic differentiation between subpopulations (0.094), while Hedrick's G''_{ST} was low (0.032). While Cabo Douglas and Crater Rim are occasionally separated by a large expanse of lava flow from recent eruptions, it does not appear to be a barrier to dispersal. During sufficient periods of volcanic dormancy, small patches of vegetation

likely connect the two subpopulations, facilitating migration between the Cabo Douglas and Crater Rim localities.

Control region haplotype diversity was high at both subpopulations, with low levels of nucleotide diversity. Φ_{ST} estimates for mtDNA were low and not significant (0.0283; p= 0.074). The number of migrants estimated from mtDNA data was much higher than estimates from microsatellites (Nm= 17.157). The haplotype network indicated no shared haplotypes within or between the subpopulations. There are also no interconnected haplotypes in the network and no shared haplotypes (Fig. 20). The high number of unique mtDNA haplotypes would indicate the presence of numerous matrilines. *Nesoryzomys fernandinae* had the second longest time since population expansion (116,326 yrs), which supports the haplotype network.

Nesoryzomys fernandinae, like *N. narboroughi*, is in need of ecological study. Though it is sympatric with *N. narboroughi*, it is not necessarily under the same selective pressures as its neighbor. Though both microsatellite and d-loop data suggest *N. fernandinae* is a single panmictic population, complete surveys of the island should be undertaken to discover other possible areas of *N. fernandinae* density.

CONCLUSION

Contrary to the initial hypotheses, the species of native rodents on the Galápagos showed surprising genetic diversity. Some populations were in Hardy-Weinberg proportions. Given the importance of genetic diversity coupled with the unpredictable and unforgiving conditions on the islands, it appears these species have maintained moderate to high levels of genetic diversity allowing them to adapt to the frequently changing setting of the Galápagos. All efforts must be made to maintain the natural levels of diversity of these species.

FUTURE WORK

Laboratory Recommendations

The presence of null alleles leads to overestimates of both F_{ST} and genetic distance (Chapuis and Estoup 2007). In cases of high null allele frequencies and low levels of gene flow, F_{ST} is greatly biased, producing artificially higher values (Chapuis and Estoup 2007). Therefore, locus Nect24 should be re-optimized to reduce the frequency of null alleles in any future work. Locus Nect14 should be re-optimized for use with *N. narboroughi* due to its early promise and high polymorphism.

Given sufficient allelic diversity, a relatively low number of loci are required to achieve high allocation success (Bernatchez and Duchesne 2000). There is no significant gain in success when increasing allelic diversity beyond approximately 6 to 10 alleles per locus and the contribution of the number of loci outweighs the number of alleles in population assignments (Bernatchez and Duchesne 2000). According to these criteria, the number of loci used in the current study should have provided sufficient number of alleles and allelic richness. To increase the resolution of estimated *F*-statistics, additional loci (rather than more allelic rich loci) should be screened and optimized.

The possible temporal substructuring documented in *A. bauri* is intriguing. Additional loci should be screened to determine if there is an El Niño effect on the genetic structure of the species. Microsatellite loci designed for other *Aegialomys* or *Oryzomys* species (such as microsatellite primers designed for *O. palustris*—Wang et al. 2000) should be optimized and included in future population genetic studies (for a minimum total of 10 polymorphic loci). Additional loci should be screened or designed for the remaining 3 species (again, with a goal of a 10 polymorphic loci). Finally, all species should be subdivided by "year collected" to test for a similar temporal pattern. There have been 435 moderate to strong El Niño events in the past ~7130 yrs and the frequency and intensity of events increased ~3,100 yrs ago (Riedinger et al. 2002). This constant turnover may be a selective factor mainting genetic variation in the rodent populations. However, since the pattern since in *A. bauri* was not detected in the *Nesoryzomys* species, there may be an ecological factor for some species mitigating ENSO effects.

Additional Studies

DNA from museum samples of *Aegialomys galapagoensis* should be extracted and included in subsequent molecular systematic analyses involving the native Galápagos rodents. These samples are critical in addressing questions related to the taxonomic status of native species of *Aegialomys* (both historic and present members of the genus). If determined to be conspecific, captive breeding colonies could be utilized to "restock" the extirpated *A. galapagoensis* population on Isla San Cristobal, following implementation of eradication efforts for feral cats and invasive rodents.

Aegialomys bauri's results were surprising given the small island area (24 km^2) and lack of isolating barriers. El Niño events can greatly alter the vegetative landscape of the islands, which in turn can impact the native wildlife. Strong ENSO in the Galápagos can be catastrophic to native wildlife, while weak ENSO events impact reproduction and the population recovery rate (Boersma 1998; Vargas et al. 2006; Vargas et al. 2007). A monitoring program should be established on Isla Santa Fe that collects demographic data as well as genetic samples from the endemic species at least bi-annually. Temporal fluctuations in genetic diversity and level of heterozygosity could then be monitored and tested for correlations to El Niño events. Ecological surveys (similar to those used to study Darwin's finches; Grant and Grant 1993) should be conducted to study selected and preferred diet shifts during El Niño years for a possible correlation between intraspecies diversity and food availability. An on-going population monitoring would provide a

"safety net" in case of an unforeseen population decline/crash and would allow for an immediate response should non-native rodents be introduced to this island.

El Niño events may not have the same impact on the other native rodent species due to the geography and habitat unique to each island. For example, the southeast approach of the prevailing trade winds results in the casting of a rain shadow over La Bomba. This results in an arid habitat characterized by *Opuntia galapageia* (Gregory and MacDonald 2009). Future studies should examine the demographic and ecological changes of both *N. swarthi* and *R. rattus* in pre- and post-El Niño events. The small and isolated patch of suitable habitat for *N. swarthi* on La Bomba, in conjunction with competition with *Rattus rattus*, may impact any El Niño effects.

An in-depth study of the ecology of both *N. fernandinae* and *N. narboroughi* is needed (Fig. 22). Such studies should examine competitive interactions of the sympatric *N. fernandinae* and *N. narboroughi* via mark-recapture and resource utilization studies. Baseline information on diet, reproduction, etc. are needed prior to the implementation of future captive breeding programs. Comprehensive studies of the island should be conducted to generate a more accurate picture of the status of these species.

Figure 22. Photo of *Nesoryzomys fernandinae* (left) and *Nesoryzomys narboroughi* (right) from Isla Fernandina (Photo courtesy of Cody W. Edwards).

Finally, phylogenetic study involving the 4 extant endemic rodent species and possible candidate South American progenitor species (e.g. *Aegialomys xanthaeolus, Oryzomys couesi, Oligoryzomys fulvescens, Ol. longicaudatus, Nectomys squamipes, N. apicalis, Holochilus brasiliensis, H. sciureus, Pseudoryzomys simplex,* and representatives of *Neacomys, Microryzomys,* or *Oecomys*—Smith and Patton 1999; Weksler 2003) would aid in addressing these and other critical issues. Candidate markers, which have been used successfully to resolve the evolutionary relationships among other species of rodents, include: cytochrome *b*, beta fibrinogen, and IRBP (Matocq et al. 2007; Nunome et al. 2007; Storz et al. 2007). Coalescence analyses with more complete sampling of mainland species and museum samples should be conducted

to generate a more accurate divergence estimate and time to most recent common ancestor (tMRCA).

APPLICATIONS

The 3 primary levels of biodiversity are genes, species, and ecosystems. All 3 levels must be conserved for successful conservation of biodiversity. Captive colonies can aid in conservation and population recovery despite small founder size and low genetic diversity. For example, the pink pigeon (*Columba mayeri*), endemic to Mauritius, recovered from less than 20 birds in the mid 1970s to over 350 birds in 2003 through *ex situ* breeding (Swinnerton et al. 2004).

Conservation breeding provides sources for founding new populations or provides genetic support for wild populations. Selecting founding individuals for captive breeding is an important decision. Genetic variation is both a trait of individuals and a trait of populations. Source populations should be selected to maximize genetic and adaptive diversity and captive colonies should be large enough to reduce inbreeding, a consequence of small population size. Inbreeding has been linked to higher mortality, lower fecundity, reduced mating ability, slower growth, developmental defects, greater susceptibility to disease, and reduced intra and inter-specific competitive ability (Lacy 1997). This is particularly magnified with insular species and endemic populations typically have inbreeding coefficients significantly higher than nonendemic island populations (Frankham 1998).

Allendorf and Luikart (2007) recommend a minimum of 30 and preferably 50 founders for captive breeding colonies. Genetic identification of captive colony founders is unnecessary. The assumption that captive population founders are unrelated and not inbred resulted in only a minor loss of gene diversity than when relationships of founders were known (0-2% gene diversity loss—Rudnick and Lacy 2008). A bi-directional migration of introducing captive bred individuals to the wild and supplementing captive colonies with wild individuals reduces the genetic load of the natural population even for longer-term programs (up to 50 generations) and decreases the likelihood of reduced fitness in the captive population (Theodorou and Couvet 2004). Mills and Allendorf (1996) found that the one-migrant-per-generation rule to minimize the loss of polymorphism and heterozygosity within subpopulations while allowing for divergence in allele frequencies among subpopulations is a desirable minimum, but may not be adequate for many natural populations. They suggest a minimum of 1 and maximum of 10 migrants per generation (Mills and Allendorf 1996). It is important that appropriate representatives from the different subpopulations of rodents on Isla Fernandinae are collected and care taken to avoid artificially differentiating "within species".

Captive environments can be radically different from natural habitats. The benign conditions of a captive colony (absence of predators, reliable food resources, medical treatment, etc.) relax the selective pressures found in the wild. Though the impacts of captivity may not be as severe for rodents, a captive colony must be managed so that the selective pressures closely resemble those in the wild to avoid genetic transformations

that would eventually lead to unsustainable natural populations (i.e. domestication selection—Lynch and O'Hely 2001).

Although captive breeding plays a large role in conservation programs, field based conservation and *in situ* management is vital. In order to protect the native rodents, it is necessary to employ methods that control the alien rodents with little or no impact on the native species. Bait stations can be a very effective, though costly, means of eradication (Moro 2001). Variations in foraging behavior between *Rattus rattus* and the endemic rodents of the Galápagos have been exploited to selectively control the invasive species (Phillips et al. 2007). Ambitious eradication efforts aimed at the 3 species of introduced rodent (*Rattus rattus, R. norvegicus,* and *Mus musculus*) should be implemented immediately. Captive breeding programs and reintroduction plans involving the native rodent species should supplement these efforts. Hopefully, the results provided in this study will be used in the implementation of better monitoring programs and in the founding of captive breeding colonies.

APPENDIX

Parque Nacional Galápagos
Ecuador

Parque Nacional Galápagos
Propuesta de Proyecto al Parque Nacional Galápagos
PR. PT.P004.R02

Proyecto N° PC-11-06
03/05/06

Título de la Propuesta: Genética, evolución y estrategias de la conservación de roedores endémicos de las Islas Galápagos.

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En la siguiente Institución: Texas Tech University

Otros participantes en el proyecto: Robert C. Dowler

Clasificación del Proyecto: Evolución y Conservación **Se requiere colectar muestras?** SI

Inicio del Proyecto: 25/07/2006 **Finalización del Proyecto:** 25/08/2006

Sitios a visitarse: Fernandina, Santiago y Santa Fe

Institución auspiciante: George Mason University

Resumen del proyecto: Se propone conducir una investigación para determinar la variabilidad genética de los roedores nativos de Galápagos, con el antecedente del redescubrimiento de la rata arrocera de Santiago la cual se creía extinta, se han realizando esfuerzos para localizar nuevas poblaciones de roedores nativos con poco éxito. Para asegurar la conservación de las 4 especies existentes se deben realizar trabajos para comprender mejor la biología de las poblaciones de roedores. Esta información permitirá la toma de decisiones para el futuro manejo de estas poblaciones hechas bajo un contexto genético. Determinar el nivel de variabilidad permitirá tomar los apropiados pasos para mantener poblaciones viables en cada especie de roedor. Los objetivos específicos son:1) Determinar el patrón de variabilidad de microsatélites dentro de las poblaciones de Nesoryzomys narboroughi, N. fernandinae, N. swarthi y Oryzomys bauri. 2) Determinar factores evolutivos que podrían afectar la estructura de la población observada. 3) Discutir estrategias apropiadas para la conservación a largo plazo de estas especies. Se realizarán monitores y colectas en Fernandina, Santa Fe y Santiago utilizando trampas Tomahwak, las trampas serán colocadas en transectos donde se colocarán 50 trampas. Dos transectos se harán cada noche por un total de 4 a 5 noches. Luego de la captura la especie será identificada, sexada, medida y se obtendrán muestras de ADN a través de muestras de mucosa bucal y heces fecales. Ectoparásitos presentes serán colectados y preservados en etanol. Las especies introducidas serán colectadas y también se tomarán muestras de tejido. Las condiciones específicas del permiso de investigación, se encuentran contenidas en los criterios del PNG al proyecto y son de cumplimiento obligatorio.

Fecha reporte inicial: 30/10/2006

Fecha reporte final: 30/12/2006

Firma Investigador Principal

Fecha: 03/05/06

Firma del Director

Autorizado ✓ **Negado**

Sello del PNG

Figure 23. Collection Permit. A copy of the collection permit for the 2006 expedition.

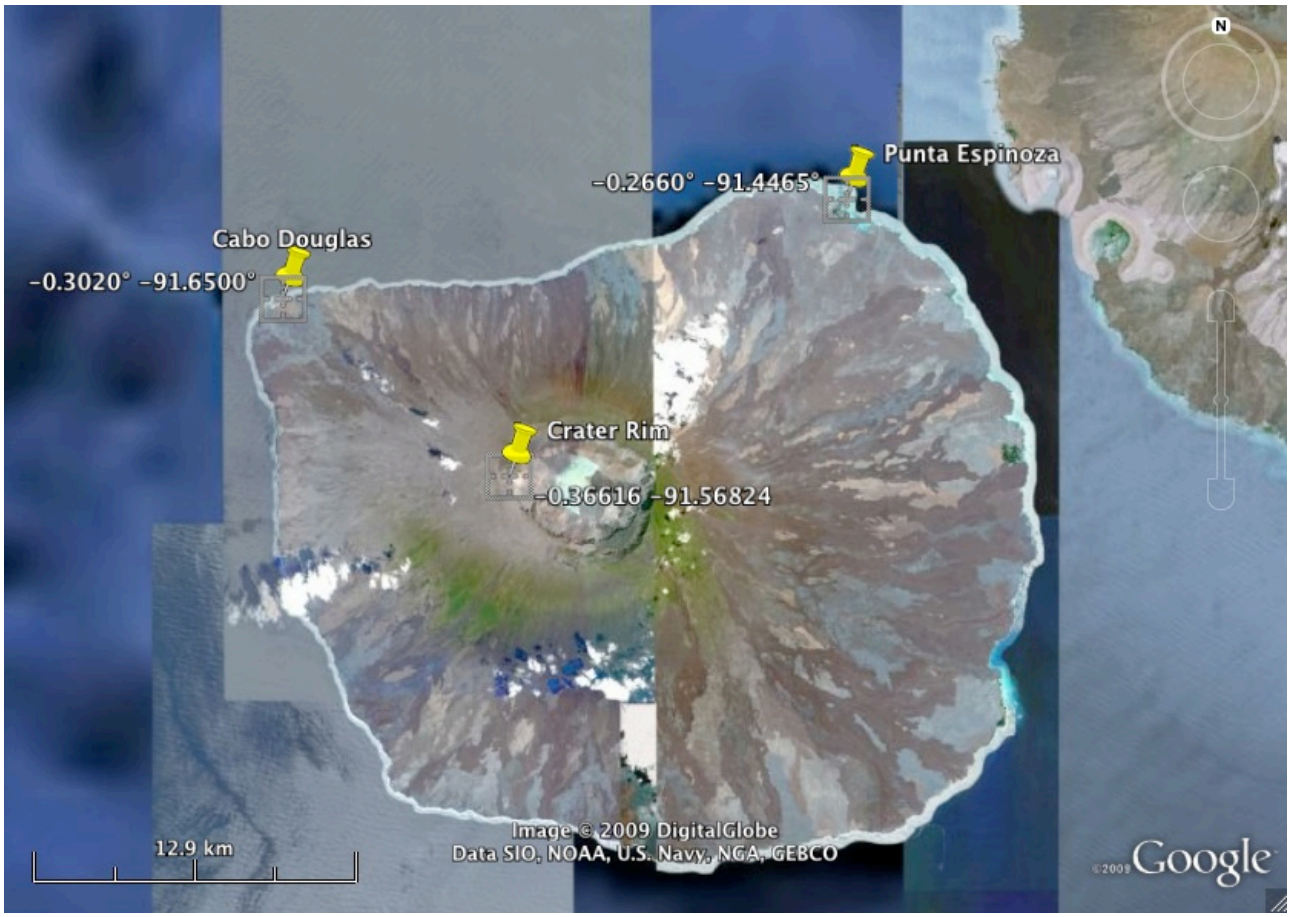

Figure 24. Sampling localities on Isla Fernandina. *Nesoryzomys fernandinae* was collected at Cabo Douglas and Crater Rim only. *Nesoryzomys narboroughi* was collected at Cabo Douglas, Crater Rim, and Punta Espinoza. (created in Google Earth)

Figure 25. Sampling localities on Isla Santiago. (created in Google Earth)

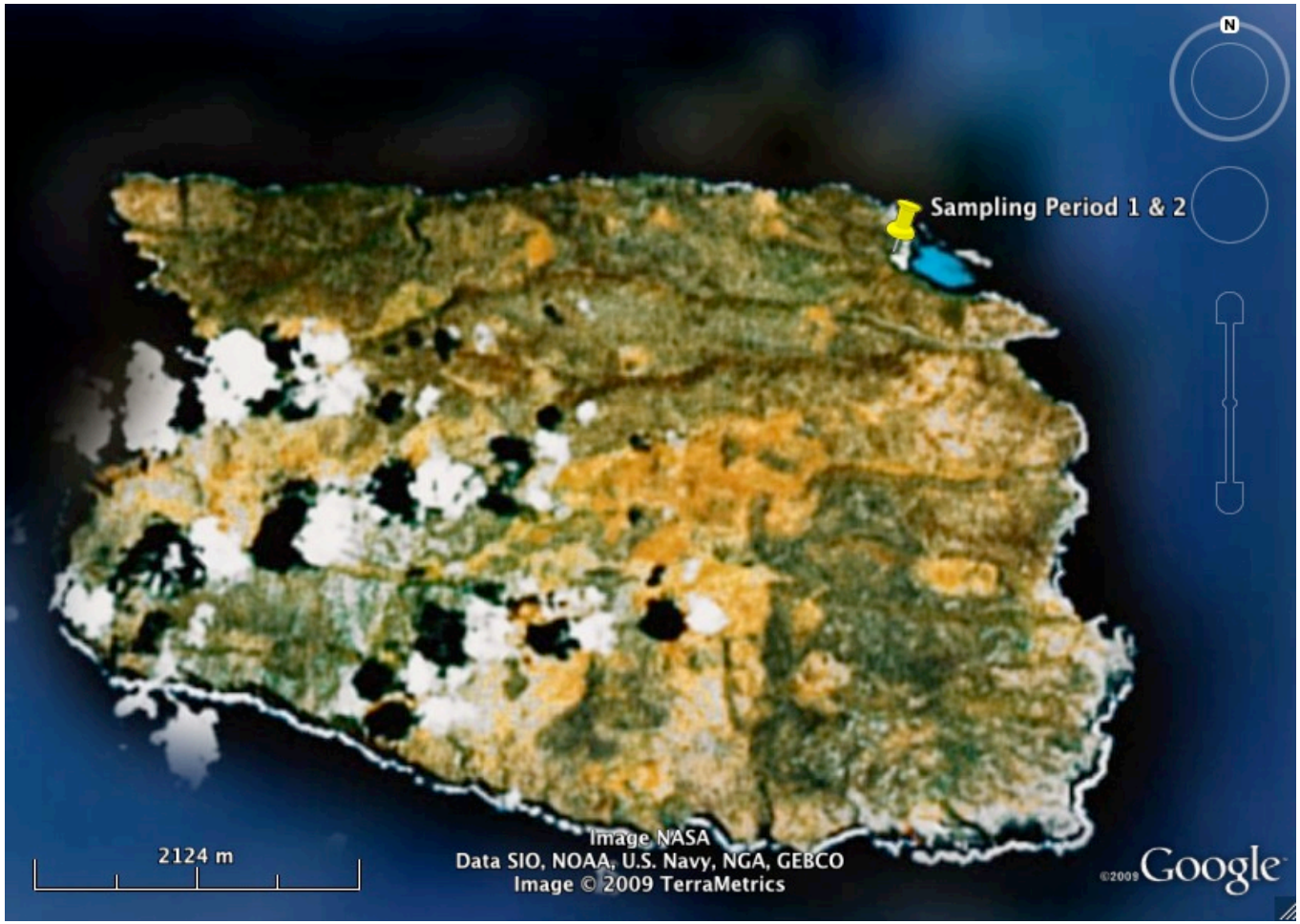

Figure 26. Sampling locality on Isla Santa Fe. (created in Google Earth)

Table 15. Voucher specimen numbers for tissues on loan from Angelo State Natural History Collection (ASNHC)

ASNHC #	Genus	species	ASNHC #	Genus	species
9994	*Oryzomys*	*bauri*	10595	*Nesoryzomys*	*narboroughi*
9997	*Oryzomys*	*bauri*	10596	*Nesoryzomys*	*narboroughi*
9990	*Oryzomys*	*bauri*	10585	*Nesoryzomys*	*narboroughi*
9998	*Oryzomys*	*bauri*	10586	*Nesoryzomys*	*narboroughi*
9991	*Oryzomys*	*bauri*	10587	*Nesoryzomys*	*narboroughi*
9995	*Oryzomys*	*bauri*	10588	*Nesoryzomys*	*narboroughi*
9999	*Oryzomys*	*bauri*	10589	*Nesoryzomys*	*narboroughi*
9992	*Oryzomys*	*bauri*	10590	*Nesoryzomys*	*narboroughi*
9996	*Oryzomys*	*bauri*	8668	*Nesoryzomys*	*narboroughi*
10613	*Oryzomys*	*bauri*	8669	*Nesoryzomys*	*narboroughi*
10614	*Oryzomys*	*bauri*	8670	*Nesoryzomys*	*narboroughi*
10615	*Oryzomys*	*bauri*	8671	*Nesoryzomys*	*narboroughi*
10616	*Oryzomys*	*bauri*	8673	*Nesoryzomys*	*narboroughi*
10617	*Oryzomys*	*bauri*	8674	*Nesoryzomys*	*narboroughi*
10618	*Oryzomys*	*bauri*	8675	*Nesoryzomys*	*narboroughi*
10619	*Oryzomys*	*bauri*	8672	*Nesoryzomys*	*narboroughi*
10620	*Oryzomys*	*bauri*	8666	*Nesoryzomys*	*narboroughi*
10609	*Oryzomys*	*bauri*	8667	*Nesoryzomys*	*narboroughi*
10610	*Oryzomys*	*bauri*	10002	*Nesoryzomys*	*swarthi*
10611	*Oryzomys*	*bauri*	10001	*Nesoryzomys*	*swarthi*
10612	*Oryzomys*	*bauri*	10003	*Nesoryzomys*	*swarthi*
11189	*Oryzomys*	*bauri*	11577	*Nesoryzomys*	*swarthi*
11188	*Oryzomys*	*bauri*	10597	*Nesoryzomys*	*swarthi*
10579	*Nesoryzomys*	*fernandinae*	10598	*Nesoryzomys*	*swarthi*
10580	*Nesoryzomys*	*fernandinae*	10599	*Nesoryzomys*	*swarthi*
10581	*Nesoryzomys*	*fernandinae*	10600	*Nesoryzomys*	*swarthi*
10582	*Nesoryzomys*	*fernandinae*	10601	*Nesoryzomys*	*swarthi*
10583	*Nesoryzomys*	*fernandinae*	10602	*Nesoryzomys*	*swarthi*
10573	*Nesoryzomys*	*fernandinae*	10603	*Nesoryzomys*	*swarthi*
10574	*Nesoryzomys*	*fernandinae*	10604	*Nesoryzomys*	*swarthi*
10575	*Nesoryzomys*	*fernandinae*	10605	*Nesoryzomys*	*swarthi*
10577	*Nesoryzomys*	*fernandinae*	10606	*Nesoryzomys*	*swarthi*
10578	*Nesoryzomys*	*fernandinae*	10607	*Nesoryzomys*	*swarthi*
8665	*Nesoryzomys*	*fernandinae*	10608	*Nesoryzomys*	*swarthi*
8664	*Nesoryzomys*	*fernandinae*			
8660	*Nesoryzomys*	*fernandinae*			
8663	*Nesoryzomys*	*fernandinae*			
8661	*Nesoryzomys*	*fernandinae*			
8662	*Nesoryzomys*	*fernandinae*			
10591	*Nesoryzomys*	*narboroughi*			
10592	*Nesoryzomys*	*narboroughi*			
10593	*Nesoryzomys*	*narboroughi*			
10594	*Nesoryzomys*	*narboroughi*			

Table 16. Population differentiation and genetic diversity in comparable studies. Both molecular markers used in the current study are represented (mtDNA and microsatellites).

mtDNA **Species**	**Haplotype Diversity *h***	**Nucleotide Diversity π**	**F_{ST}**	**Author**
Southern Plains woodrat *Neotoma micropus*	0.974	0.008	0.205	Mendez-Harcelode et al. 2005
Gray mouse lemur *Microcebus murinus*	0.153	0.041 ± 0.003	Not given	Wimmer et al. 2002
Southern Plains woodrat *Neotoma micropus*	0.964	0.007	0.061	Mendez-Harclerode et al. 2007
Australian bushrat *Rattus fuscipes greyii*	0 to 0.889	0 to 0.017	Not given	Hinten et al. 2003
Red Kangaroo *Macropus rufus*	0.994	0.0357	0.180	Clegg et al. 1998
Sea Otter *Enhydra lutris*	0.412	0.001 ± 0.0003	0.077 to 0.466	Larson et al 2006
Brown Lemming *Lemmus trimucronatus*	0.250	0.210	0.534	Ehrich et al. 2001
Collared Lemming *Dicronstonyx groenlandicus*	0.150	0.060	0.011	Ehrich et al. 2001
Steller sea lion *Eumetopias jubatus*	0.927		0.050	Bickham et al. 1996
Nesoryzomys swarthi	1.000 ± 0.008	0.019	0.165	Current study
Nesoryzomys narboroughi	1.000 ± 0.005	0.009	0.009	Current study
Nesoryzomys fernandinae	1.000 ± 0.029	0.022	0.028	Current study
Oryzomys bauri	1.000 ± 0.003	0.009	0.007	Current study

Table 16. (continued)

Microsatellite Species	Number of loci	F_{ST}	Other Indices Reported	Author
Southern Plains woodrat *Neotoma micropus*	5	0.011	$R_{ST} = 0.007$	Mendez-Harclerode et al. 2007
Domestic dog *Canis familiaris*	8	0.154	$G_{ST} = 0.1537$	Kim et al. 2001
Norway rat *Rattus norvegicus*	7	0.008 to 0.649		Calmet et al. 2001
Australian bushrat *Rattus fuscipes greyii*	6	0.110 to 0.840	$R_{ST} = 0.12$ to 0.99	Hinten et al. 2003
Norway rat *Rattus norvegicus*	8	0.410		Abdelkrim et al. 2005
Coues' rice rat *Oryzomys couesi cozumelae*	5	-0.066 to 0.069	$R_{ST} = -0.031$ to 0.233	Vega at al. 2007
Sea Otter *Enhydra lutris*	7	0.049 to 0.183		Larson et al 2006
Brown Lemming *Lemmus trimucronatus*	4	0.124		Ehrich et al. 2001
Collared Lemming *Dicronstonyx groenlandicus*	4	0.047		Ehrich et al. 2001
Black footed ferret *Mustela nigripes*	24	0.570 ± 0.150		Wisely et al 2000
Northern Idaho ground squirrel *Spermophilus brunneus brunneus*	5	0.167		Gavin et al. 1999
Allegheny woodrat *Neotoma magister*	11	0.050 to 0.460		Castleberry et al. 2002
Red-backed vole *Clethrionomys gapperi*	2	0.070		Reese et al. 2001
Nesoryzomys swarthi	4	-0.012	$G''_{ST} = -0.038$ $R_{ST} = -0.019$	Current study
Nesoryzomys narboroughi	4	0.071	$G''_{ST} = 0.123$ $R_{ST} = 0.059$	Current study
Nesoryzomys fernandinae	5	0.013	$G''_{ST} = 0.032$ $R_{ST} = 0.094$	Current study
Oryzomys bauri	4	0.158	$G''_{ST} = 0.241$ $R_{ST} = 0.034$	Current study

Table 17. Primers and annealing temperatures used in the current study.

Primer name	Region	Nucleotide Sequence 5'-3'	Annealing Temp. ºC	Species successfully amplified	Reference
Nect08	microsatellite	**F-**GTGGATGGATTCATGTGATCTG **R-**CAGACAGGGTCTCACTAAGTTGC	55 ºC	*Nesoryzomys sp*., *Aegialomys bauri*	Maroja et al. 2003
Nect12	microsatellite	**F-**CTCCCTTCCCTCAATTTGCTGAGT **R-**ACATGTGCAAAGCATGAAAATGGA	55 ºC * 60 ºC **	**Aegialomys bauri* ***Nesoryzomys sp*.	Almeida et al. 2000
Nect13	microsatellite	**F-**TATCTGCTCAACATTCCAGGGT **R-**TGGAGTAAGATGCTCAGAGTTG	52 ºC	*Nesoryzomys sp*., *Aegialomys bauri*	Maroja et al. 2003
Nect14	microsatellite	**F-**CAGGCGATTTACACAAAAGAAT **R-**CACTGAGCCATCTATCCAGTTC	42 ºC	*Nesoryzomys sp*., *Aegialomys bauri*	Almeida et al. 2000
Nect15	microsatellite	**F-**AGGAAATGCTTGAACTGACC **R-**GACTCCTGATGTTGAACTGACC	51 ºC	*Aegialomys bauri* only	Almeida et al. 2000
Nect17	microsatellite	**F-**TCCCTGGTTATCATACTTGAGG **R-**GACAGTTCTCACTTTTCCATGG	—	None in this study	Maroja et al. 2003
Nect18	microsatellite	**F-**CTCTTTGAGGCCACTTCATTA **R-**GAACTAACATTTGACTCCTCCAG	60 ºC	*Nesoryzomys spp*. only	Almeida et al. 2000
Nect19	microsatellite	**F-**CCAAATGGTGCCTAAAAATCAG **R-**TAGTAGAGAGCAACCAAAGGCC	—	None in this study	Maroja et al. 2003
Nect23	microsatellite	**F-**CTACCTCCAAAACAGAGAAAGG **R-**CTGATTTCTGTGTATGATTGAGAT	52 ºC	*Nesoryzomys sp*., *Aegialomys bauri*	Maroja et al. 2003
Nect24	microsatellite	**F-**CTTCTGCCCTCCACAAATGATT **R-**GTAGCAACTGCGTAACTTCCCC	55 ºC	*Nesoryzomys sp*., *Aegialomys bauri*	Maroja et al. 2003
Nect28	microsatellite	**F-**AGGAGAAAACCTGTATGCCATG **R-**GTTTCTTCTTGCTGACCATGAGG	52 ºC	*Nesoryzomys sp*., *Aegialomys bauri*	Almeida et al. 2000
Nect29	microsatellite	**F-**CAAATGTCCTCTGGTCTTCAC **R-**CAACATTAGAGAAATTCAGGGC	48 ºC	*Nesoryzomys sp*., *Aegialomys bauri*	Maroja et al. 2003
Oligo	d-loop (external)	**F-**TGAATTGGAGGACAACCAGT **R-**AAGGCTAGGACCAAACCT	52 ºC	*Nesoryzomys sp*., *Aegialomys bauri*	Gonzalez Ittig et al. 2002
GIF	d-loop (internal)	**F-**CCACTACCAGCACCCAAAGCTG	58 ºC	*Nesoryzomys sp*., *Aegialomys bauri*	Current project
GIREV	d-loop (internal)	**R-**GGTTGTGTTGATTAATGATCC	52 ºC	*Nesoryzomys sp*., *Aegialomys bauri*	Current project

Table 18. Allele frequency per locus per subpopulation in *A. bauri*

Locus	Allele Size (bp)	Sampling Period 1 (1997)	Sampling Period 2 (2006)	Total Frequency (Weighted)
	237	0.043	*0*	0.017
	239	0.174	0.764	0.534
Nect08	241	0.543	0.014	0.220
	243	0.043	*0*	0.017
	247	0.043	0.153	0.110
	249	0.152	0.069	0.102
	233	0.174	0.042	0.093
Nect12	235	0.174	0.361	0.288
	237	0.587	0.486	0.525
	239	0.022	0.069	0.051
	241	0.043	0.042	0.042
	206	0.281	*0*	0.281
Nect14	208	0.375	*0*	0.375
	210	0.125	*0*	0.125
	212	0.219	*0*	0.219
	191	0.109	0.097	0.102
	193	0.043	*0*	0.017
	195	0.283	0.514	0.424
Nect15	201	0.065	0.042	0.051
	203	0.043	*0*	0.017
	205	0.326	0.347	0.339
	209	0.087	*0*	0.034
	213	0.022	*0*	0.008
	215	0.022	*0*	0.008
	101	*0*	0.016	0.010
Nect24	109	*0*	0.016	0.010
	115	0.941	0.969	0.959
	165	0.059	*0*	0.020

Table 19. Allele frequency per locus per subpopulation in *N. swarthi*

Locus	Allele Size (bp)	Eastern Beach	La Bomba	Total Frequency (weighted)
	211	0.235	0.286	0.267
Nect08	213	0.588	0.589	0.589
	215	0.176	0.125	0.144
	217	0.167	0.250	0.223
	221	0.167	0.066	0.098
	223	0.167	0.145	0.152
	225	*0*	0.079	0.054
Nect12	231	0.250	0.250	0.250
	239	0.028	0.053	0.045
	243	*0*	0.053	0.036
	245	0.028	0.013	0.018
	247	0.194	0.092	0.125
	194	*0*	0.444	0.444
	198	*0*	0.056	0.056
	200	*0*	0.167	0.167
Nect14	204	*0*	0.056	0.056
	208	*0*	0.056	0.056
	210	*0*	0.167	0.167
	216	*0*	0.056	0.056
	92	*0*	0.045	0.030
	95	0.029	*0*	0.010
	98	0.059	0.450	0.050
Nect18	101	0.382	0.364	0.370
	104	0.029	*0*	0.010
	107	0.471	0.515	0.500
	122	*0*	0.030	0.020
	204	0.029	*0*	0.010

Table 19. (continued)

Locus	Allele Size (bp)	Eastern Beach	La Bomba	Total Frequency (weighted)
	113	*0*	0.014	0.009
	123	0.056	0.028	0.037
	125	0.083	0.056	0.065
	127	0.194	0.222	0.213
	129	0.111	0.056	0.074
Nect24	131	0.194	0.236	0.222
	133	0.111	0.264	0.213
	135	0.139	0.097	0.111
	137	0.056	*0*	0.019
	141	*0*	0.028	0.019
	143	0.028	*0*	0.009
	145	0.028	*0*	0.009

Table 20. Allele frequency per locus per subpopulation in *N. narboroughi*

Locus	Allele Size (bp)	Cabo Douglas	Punta Espinoza	Crater Rim	Total Frequency (weighted)
	227	0.010	*0*	*0*	0.008
	229	0.480	0.278	0.300	0.438
	231	0.088	0.056	0.200	0.092
Nect08	233	0.235	0.333	0.500	0.269
	235	0.127	0.167	*0*	0.123
	237	0.010	0.111	*0*	0.023
	239	0.049	*0*	*0*	0.038
	243	*0*	0.056	*0*	0.008
	219	0.009	*0*	*0*	0.007
Nect12	245	*0*	0.278	*0*	0.036
	247	0.200	0.278	0.250	0.214
	253	0.791	0.222	0.750	0.714
	255	*0*	0.222	*0*	0.029
Nect14	178	1	1	1	1
	85	0.018	*0*	*0*	0.014
	107	0.009	*0*	*0*	0.007
	125	0.009	*0*	*0*	0.007
Nect18	149	0.036	0.056	*0*	0.036
	152	0.309	*0*	*0*	0.243
	155	0.191	0.278	0.500	0.229
	158	0.155	0.056	0.083	0.136
	162	0.273	0.611	0.250	0.314
	165	*0*	*0*	0.167	0.014
	137	0.012	*0*	*0*	0.009
	139	0.035	*0*	0.167	0.045
	141	0.023	0.143	*0*	0.036
Nect24	143	0.267	0.357	0.250	0.277
	145	0.140	0.143	0.250	0.152
	147	0.488	0.286	0.333	0.446
	149	*0*	0.071	*0*	0.009
	153	0.035	*0*	*0*	0.027

Table 21. Allele frequency per locus per subpopulation in *N. fernandinae*

Locus	Allele Size (bp)	Cabo Douglas	Crater Rim	Total Frequency (weighted)
	213	0.267	0.200	0.229
	215	0.333	0.350	0.343
	217	0.167	0.075	0.114
Nect08	219	0.067	0.050	0.057
	229	*0*	0.025	0.014
	231	0.100	0.100	0.100
	233	*0*	0.125	0.071
	235	*0*	0.050	0.029
	237	*0*	0.025	0.014
	241	0.067	*0*	0.029
	207	0.031	0.048	0.041
	223	0.438	0.214	0.311
Nect12	225	0.094	0.381	0.257
	227	0.250	0.167	0.203
	229	0.094	0.071	0.081
	231	*0*	0.095	0.054
	237	0.094	0.024	0.054
	188	0.818	0.647	0.714
Nect14	190	0.182	0.353	0.286
	116	*0*	0.048	0.029
	122	0.464	0.524	0.500
	125	0.464	0.381	0.414
Nect18	128	0.036	0.048	0.043
	131	0.036	*0*	0.014

Table 21. (continued)

Locus	Allele Size (bp)	Cabo Douglas	Crater Rim	Total Frequency (weighted)
	103	*0*	0.107	0.058
	111	*0*	0.071	0.038
	113	0.042	0.107	0.077
	121	0.167	0.107	0.135
	123	0.375	0.357	0.365
Nect24	125	*0*	0.143	0.077
	127	0.042	0.071	0.058
	143	0.083	*0*	0.038
	145	0.208	0.036	0.115
	147	0.042	*0*	0.019
	149	0.042	*0*	0.019

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