Hybrid zone genetics and within-island diversity of the gecko *Tarentola boettgeri*

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The following figures and appendix have been omitted on request of the university –

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Abstract

This study builds on a previous study that demonstrates the existence of deep mitochondrial lineages in the gecko *Tarentola boettgeri* within Gran Canaria (Gübitz et al. 2005). Here, I identified and analyzed the area where the two most divergent mitochondrial lineages meet. The primary aim was to examine how geographical structuring of mtDNA has been maintained after secondary contact. MtDNA analyses used a 608 basepair fragment of the cytochrome b (cyt b) gene from 389 individuals sampled from 14 populations along a 32 km southeast (SE) transect across Gran Canaria. It revealed a low degree of mtDNA admixture and negligible gene flow across the contact zone. This led to the hypothesis that reproductive barriers may have formed between populations from different mtDNA lineages. Analyses of seven body dimension and scalation characters revealed that spatial patterns of morphological changes were not associated with the transition in mtDNA lineage frequency across the transect. This contrasted with another lizard species on the same island, *Chalcides sexlineatus*, in which phylogeography and morphology are highly correlated. This study identified ten unique microsatellite markers in *T. boettgeri*. Like morphology, analyses of these microsatellites did not reveal a pronounced spatial pattern of differentiation in the nuclear genome. These results appear to reject the hypothesis of a physical or genetic barrier to reproduction. Studies of microsatellites also suggested that *T. boettgeri* is a low dispersal species and this might explain the persistence of mtDNA contact zone. However, evidence of concordant spatial patterns between divergence in the nuclear genome and morphology was detected. The discordant spatial patterns of mitochondrial and nuclear genotype frequencies do not appear to be explained by sex-biased gene flow, and are difficult to understand because of expected interactions between the two genomes. Thus, further investigation is suggested to allow clarification of the causes of mito-nuclear discordance in *T. boettgeri*.

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1.1 The Canary Islands and their importance in evolutionary studies

1.1.1 Geology of the Canary Islands

The Canary Archipelago is situated to the northwest of Africa, between 27°38' and 29°25' latitude and 13°20' and 18°9' longitude. The archipelago consists of seven major islands, forming a ~500 km island chain (Figure 1). The formation of the archipelago began in the Senonian (~ 80 Ma) (Balogh et al. 1999). The first island (Fuerteventura) emerged from the seafloor during the last 20 million years (Mys), followed by westward propagation of the island chain (Figure 1) (Hoernle and Schmincke 1993). Each island originated from independent subaerial volcanic eruptions and the islands are separated from one another by deep sea channels (Schmincke and Sumita 1998).

Figure 1. Map showing the Canary Islands. Numbers in brackets show approximate age of the islands (Guillou et al. 1996; Schmincke and Sumita 1998; Anguita and Hernán 2000). (The satellite image was created using Google Earth version 5.2)

1.1.2 Ecology of the Canary Islands

The Canary Islands have a subtropical climate with daily temperatures ranging from approximately 18°C to 25°C at sea level (Fernandopulle 1976). Each island is ecologically heterogeneous due to its altitude and the influence of the north-eastern tradewinds (see Figure 2). In the islands with peaks > 800 m a.s.l., the environmental variation also depends quite considerably on latitudinal variation. Cloud condensation on the windward northern slope brings higher humidity to the northern parts of the islands. On the other hand, the southern leeward parts of the islands are extremely dry.

Figure 2. A schematic representation of the vegetational zones of the Canary Islands in relation to altitude and exposure to tradewinds (from Juan et al. 2000). Cloud layer formed at the windward northern slope as a result of wind exposure is also shown.

1.1.3 The Canary Islands and evolutionary studies

The Canary Islands have become a model region for evolutionary biology over the last 25 years due to their well-documented geographical history and high levels of biological diversity (Machado et al. 1985; Oromí et al. 1991; Juan et al. 2000; Carrascal et al. 2008; Reyes-Betancort et al. 2008). There has been an increasing amount of research carried out on colonization and evolution of endemic species in the archipelago (Figure 3).

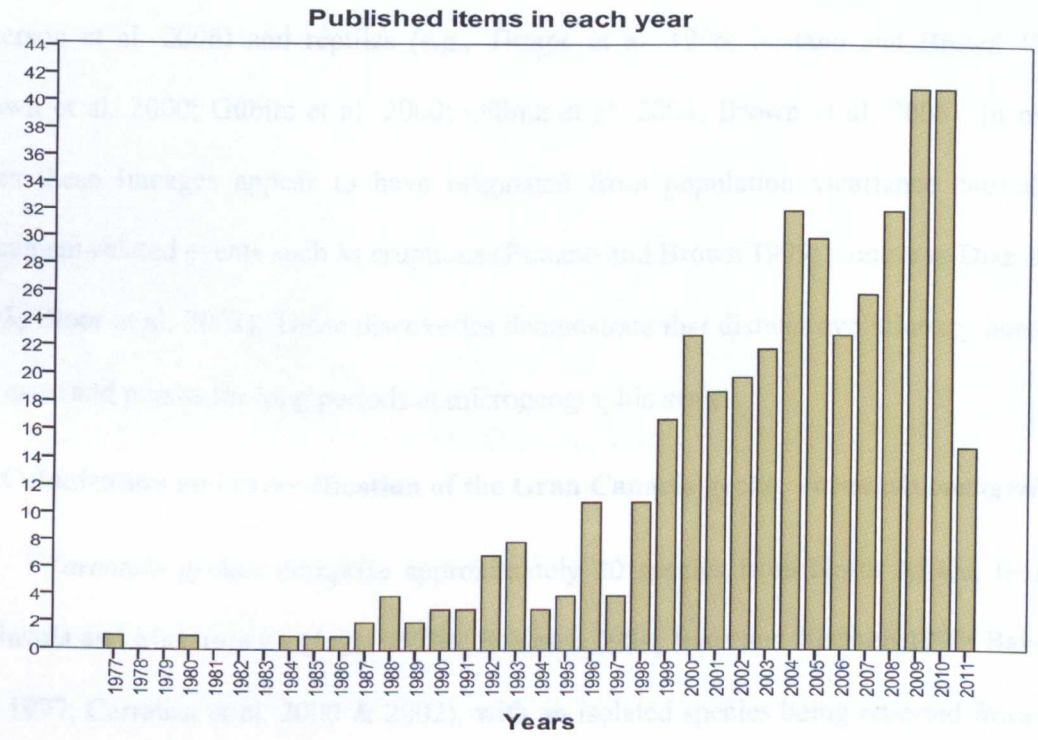


Figure 3. Growth in publications since 1997 containing all of the following key words: "Canary, Islands, Evolution, Biology, and Genetics". Information obtained from the Science Citation Index (SCI, source: Web of Sciences®).

The origin and diversification of many Canary Island endemics have been examined using mtDNA and other molecular markers, e.g., plants (Francisco-Ortega et al. 2000), birds (Kvist et al. 2005), insects (De La Rúa et al. 2001; Emerson and Oromí 2005), bats (Pestano et al. 2003) and reptiles (Gonzalez et al. 1996; Nogales et al. 1998). A principal focus of these studies has been the original colonization from continental areas and subsequent between-island dispersal (e.g., Brown and Pestano 1998; Widmer et al. 1998; Vogel et al. 2003; Cox et al. 2010). However, many studies have also uncovered significant within-island diversification, with deep intra-specific evolutionary lineages being described in insects (e.g., Juan et al. 1998; Emerson et al. 2000; Rees et al. 2001; Emerson et al. 2006) and reptiles (e.g., Thorpe et al. 1996; Pestano and Brown 1999; Brown et al. 2000; Gübitz et al. 2000; Gübitz et al. 2005; Brown et al. 2006). In many cases these lineages appear to have originated from population vicariance caused by volcanism-related events such as eruptions (Pestano and Brown 1999; Contreras-Díaz et al. 2003; Bloor et al. 2008). These discoveries demonstrate that distinct evolutionary lineages can arise and persist for long periods at microgeographic scales.

1.2 Colonization and diversification of the Gran Canaria gecko, *Tarentola boettgeri*

Tarentola geckos comprise approximately 20 species from North Africa, Iberian Peninsula and Macaronesia (Joger 1984a; Schleich 1984; Báez and Biscoito 1993; Baha el Din 1997; Carranza et al. 2000 & 2002), with an isolated species being reported from the Caribbean (Sprackland and Swinney 1998). Four *Tarentola* species have been described from the Canary Islands based on immunological and morphological studies (Joger 1984b; 1984c), which has been supported by analyses of mitochondrial DNA (mtDNA) (Nogales et al. 1998; Carranza et al. 2000). Only one species is present on each island.

Figure 4. Distribution of *Tarentola* species on the Canary Islands: *T. angustimentalis* (Steindachner 1891) from Lanzarote & Fuerteventura, *T. gomerensis* (Joger & Bischoff, 1983) from La Gomera, *T. delalandii* (Duméril & Bibron 1836) from Tenerife and La Palma, and *T. boettgeri* (Steindachner 1891) including the subspecies *T. b. hierrensis* and *T. b. bischoffi* (Joger & Bischoff 1983) from Gran Canaria, El Hierro, Selvages. Arrows indicate likely colonization events by *T. boettgeri* (Carranza et al. 2000). (The satellite image was created using Google Earth version 5.2)

Tarentola boettgeri is the only native species of gecko found on the island of Gran Canaria. It is a small, predominantly nocturnal insectivorous lizard. It is distributed throughout the island, from sea level up to altitudes of 1650 m, and is particularly abundant in xeric areas in the south (Brown 1996). Phylogenetic analyses of *Tarentola* geckos (based on *cytochrome b*, 12S rDNA and *c-mos* sequences) revealed that *T. boettgeri* colonized the island around 5.3 – 6.7 mya (Carranza et al. 2002). *T. boettgeri* has also colonized the Selvage islands, some 200 km to the north, and also the island of El Hierro in the west of the archipelago. These two island forms have been classified as two different subspecies (Figure 4). Three possible sources of colonization have been proposed: North Africa, the Selvage Islands and the Madeira Archipelago (Joger 1984c; Nogales et al. 1998; Carranza et al. 2000). However, local climatic and sea conditions (i.e., direction of the tradewinds and the Canary Current) seem to support an original colonization from the Madeira Archipelago (Joger 1984c; Carranza et al. 2000) (Figure 4).

T. boettgeri shows extraordinarily high mtDNA diversity within Gran Canaria. Two major mitochondrial lineages (designated A and B) have been reported (Nogales et al. 1998; Carranza et al. 2000; Carranza et al. 2002; Gübitz et al. 2005). MtDNA divergence between these lineages is 10.9%, based on the cytochrome b gene (Gübitz et al. 2005). The A lineage comprises 3 major sublineages, which Gübitz et al. (2005) described as A1, A2 and A3. Lineage B comprises B1 and B2 sublineages (Gübitz et al. 2005). Average mtDNA divergences among sublineages within A and B are 4.6-8.1% (Gübitz et al. 2005). (This thesis builds on Gübitz' work and for clarity I will use the lineage names used in his work throughout this thesis.) Geographical distributions of the lineages are shown in Figure 5.

Figure 5. Broad geographical distributions of the main *T. boettgeri* mtDNA lineages within Gran Canaria (redrawn from Gübitz et al. 2005). Two main lineages, A and B, are distributed mainly in the Eastern and Western sides of the island, respectively (with the exception of sublineage A3 that is distributed across the island.)

It seems likely that these deep mtDNA clades have originated *in situ* rather than due to multiple colonizations and, at least in part, result from volcanic activity over the last 4 Ma (Gübitz et al. 2005). The divergence of the main A and B clades appears to predate the Roque Nublo cycle of volcanic eruptions on Gran Canaria, with Gübitz et al. (2005) estimating a divergence time of 4.6-11 mya. Divergence within A and B appears to correspond to eruptions during the Roque Nublo cycle which caused population vicariance due to major lava flows within the island. This is assumed to have occurred less than 4.3 mya. Another species of lizard, the Gran Canarian skink *Chalcides sexlineatus*, shows within-island mtDNA lineages that appear to have originated in a similar way (Pestano and Brown 1999), and even shows phylogeographic breaks that are coincident with some of the breaks in *T. boettgeri.* Evidence supporting volcanism as a cause of within-island mtDNA phylogeography has also been found in other species on the Canary Islands (e.g, Thorpe et al. 1996; Emerson et al. 1999; Contreras-Díaz et al. 2003; Anderson et al. 2009). In summary, all current evidence strongly supports the theory that within-island mtDNA lineages originated from populations being isolated by major volcanic events. Subsequent range expansion by these populations has then led to the areas of secondary contact (which I will also call "phylogeographic breaks").

Despite the clear geographical structuring and depth of the mtDNA lineages, the geographical variation in morphology of *T. boettgeri* appears to show quite a different pattern that is associated with ecological variation in the island (Gübitz et al. 2005). This contrasts with some other Canarian reptiles which show concordant spatial patterns of phylogeography and morphological variation, e.g., *Chalcides* skinks (Pestano and Brown 1999; Brown et al. 2000). Neverthless, the finding of a close relationship between within-island morphological variation and habitat variation has been reported for several Canary Island lizards (e.g., Brown et al. 1993; Thorpe et al. 1996; Bloor and Brown 2005) as well

as for lizards in other archipelagos (Thorpe and Malhotra 1996). This has led some authors to forward adaptative explanations of habitat-associated variation in morphology. Gübitz et al. (2005) concluded that adaptation to environment was likely to have overidden historical effects on the morphological variation of *T. boettgeri*.

1.3 Maintenance of genetic structure in *T. boettgeri*

T. boettgeri therefore makes an interesting model to examine how such clear geographical structuring might be maintained between these very ancient mtDNA lineages (Figure 5). The structuring is clearly suggestive of barriers to mtDNA flow between populations, but there are no clear geographical barriers between populations that could account for this. Possible explanations for low mitochondrial gene flow are low vagility and extreme philopatry of female *Tarentola* (Gübitz et al. 2000; 2005). However, there is no scientific evidence to support these explanations.

The degree of mtDNA divergence (based on *cytochrome b*) within *T. boettgeri* is remarkably high (10.9%) and comparable to levels of interspecific divergence among other reptiles (~ 12%) (Avise et al. 1998; Johns and Avise 1998). One hypothesis could be that the mtDNA lineages described by Gübitz et al. (2005) represent more than one species and that geographical structuring is maintained by a lack of gene flow between species.

Under a unified species concept (De Queiroz 2007), species are regarded as separately evolving metapopulation lineages, and their delimitation can be achieved by demonstrating a lack of gene flow between populations. An examination of gene flow among *T. boettgeri* populations in these contact zones is therefore critical to understanding how the geographical structuring is maintained. Many contact zone studies have reported different rates of mtDNA gene flow into populations on either site of the contact zones, depending on factors such as degree of divergence and rate of dispersal (Fleischer et al.

1991; Quesada et al. 1998; Brown et al. 2006; Pedall et al. 2011). If mtDNA lineages of *T. boettgeri* reflect the presence of two species it is predicted that (i) restrictions to nuclear gene flow and (ii) coincident morphological differences should be observed along the contact zone, as reported in other species (Szymura and Barton 1986; Helbig et al. 2001).

It was not possible for the original study to examine these hypotheses due to the fact that it was based on only 48 specimens from across the island. Hence, nothing is currently known about the genetic composition of the populations where the mtDNA lineages meet. In addition, there have been no attempts to investigate patterns of nuclear genetic variation among populations.

Explanations of patterns of mtDNA phylogeography can be largely divided into three groups: (i) natural selection, (ii) genetic incompatibility, and (iii) demographical factors. The effects of ecological variation on mtDNA distribution (and morphology) were reported on an *Anolis* lizard in a Caribbean island (Malhotra and Thorpe 1994). Natural selection may contribute to mtDNA distribution in two major ways. First, selective pressures favour some haplotypes in certain environments (Mishmar et al. 2003). Second, natural selection influences genetic structure by creation of morphological differences, which can affect interbreeding (Davidson 2000; Pinceel et al. 2004). The first case is unlikely to explain the main mtDNA pattern in *T. boettgeri* as the areas of secondary contact between the major lineages is environmentally uniform. The latter explanation is possible only if distinct morphological traits are associated with the different mtDNA lineages of *T. boettgeri* which may be detected if historical selective pressures have led to morphological differences (Davidson 2000).

Genetic incompatibility between parental genomes may influence the distribution of mtDNA due to mito-nuclear interactions (Thulin and Tegelström 2002; Ballard and Rand 2005). Several mitochondrial genes, particularly those involved in the electron

transport chain, co-function and have co-evolved with their nuclear gene counterparts (Grossman et al. 2004; Rand et al. 2004). In addition, it was reported in many species of insects that different mtDNA haplotypes within a species reduced fitness on individuals with different nuclear genomes (Turelli et al. 1992; Ballard and Whitlock 2004). Although mito-nuclear co-evolution has never been studied in reptiles, it could influence the pattern of mtDNA distribution in *T. boettgeri*. The process would require there to be more than one nuclear lineage and also co-evolved mito-genomic interactions so that a mtDNA lineage in the "wrong" nuclear background would lead to inviable offspring. If this hypothesis is correct, nuclear loci should differ among different individuals with distinct mtDNA lineages, and concordant patterns of nuclear and mitochondrial distribution would be expected across the contact zone, as observed in other species (Bernardi et al. 1993; Sequeira et al. 2005).

Demographic factors, such as low vagility have contributed to maintenance of genetic structure in some species (Arnaud and Laval 2004; Thacker and Hadfield 2000). In reptiles with low vagility and high philopatry, gene flow is much lower between populations and geographical structuring is more pronounced (Garcia-Paris et al. 2000; Hasbún et al. 2005; Barbanera et al. 2009). Although this has not been studied, *Tarentola* geckos are usually considered to be a philopatric species (Thorpe 1991; Nogales et al. 1998; Gübitz et al. 2005). In order to examine the effect of low vagility on the phylogeographic pattern large sample sizes are needed to enable estimation of gene flow between populations.

1.4 General Aim

The aim of this thesis was to examine the population genetics of the areas where the ancient mtDNA lineages of *T. boettgeri* on the island of Gran Canaria come into secondary contact in an attempt to address how this within-island genetic structuring is

maintained. To achieve this, *T. boettgeri* will be sampled intensively over the phylogeographical break between the major lineages (Gübitz et al. 2005) in an attempt to provide additional resolution to identify the contact zones. Morphological and molecular data (nuclear and mitochondrial DNA) will be analysed in relation to geographical location to clarify the nature of the genetic transition between the A and B population, i.e., to determine the introgression of these markers across the zone, determine levels of gene flow between populations, and determine whether the *T. boettgeri* mtDNA lineages represent different species. The results obtained from this research may help shed light on the factors that maintain the long-term stability of these contact zones and how the process of speciation is completed.

Chapter 2 Identification and analysis of secondary contact between mitochondrial DNA lineages in *T. boettgeri*

2.1 Introduction

Geographical events occurring on large (e.g., glaciations) and small scales (e.g., volcanic eruptions) have had consequences for organisms living within these areas by driving lineage differentiation (Carson et al. 1990; Taberlet et al. 1998; Hewitt 2000). Phylogeographical studies based on mtDNA markers have revealed numerous cases of apparently deep historical subdivisions within species (see reviews in Avise et al. 1987; Taberlet 1998; Avise 2001; Hewitt 2001). Many of these studies show levels of intraspecific sequence divergence that approach interspecific levels for the taxa concerned (e.g., Moritz et al. 1987; James and Moritz 2000; Johnson and Jordan 2000; Demboski and Cook 2001; Brown et al. 2002; Stuart-Fox et al. 2002). In some cases, evidence for substantial reproductive isolation have been obtained from analysis of zones of secondary contact between mtDNA lineages (e.g., Phillips et al. 2004; Berthier et al. 2006; Mallet et al. 2007; Larsen et al. 2010).

Molecular genetic data can provide support information for species delimitations (Yang and Rannala 2010). The isolation-with-migration (IM) model (Nielsen and Wakeley 2001; Hey 2006) is based on populations splitting, follow by contact with genetic exchange. If they do not exchange genes when they come back together, then this is evidence of speciation (Slatkin 1987). Intraspecific studies of species with deep mtDNA lineages have shown that when a long period of isolation has elapsed, then this will result in 1) lineage sorting, i.e., reciprocal monophyly of mitochondrial lineages and 2) substantial morphological and nuclear DNA differentiation (Matocq 2002). Intraspecific mtDNA studies frequently detect well-supported reciprocally monophyletic lineages (e.g., Masta 2000; Poulakakis et al. 2005; Gutiérrez-Rodríguez et al. 2008). When it is accompanied by concordant patterns of morphology and nuclear markers at the contact zone, it is suggested that the mtDNA lineages have diverged into distinct species. Species

status is usually assigned to populations showing coincident splits in all of these characters (e.g., Baker and Davis 1989; Alexandrino et al. 2005; Piller et al. 2008; Albert and Fernández 2009).

Although the model of divergence and subsequent genetic exchange has been the subject of many studies, spatial patterns may be more complex than this. In particular, different genetic markers may show different spatial patterns. For example, it is often observed that a change in mtDNA lineage frequencies (or phylogeographic break) does not correspond to a similar change in nuclear markers or morphology (e.g., Janzen et al. 2002; Lindell et al. 2005; McGaugh et al. 2008; Renoult et al. 2009). Cryptic species may also be found where transitions in mtDNA and/or nuclear allele frequencies are not associated with morphological changes (e.g., Ravaoarimanana et al. 2004; Dépraz et al. 2009; Oliver et al. 2009; Yan et al. 2010). Stochastic differences in the coalescent should lead to differences among loci. However, this does not always seem to explain differences in these spatial patterns.

Due to its maternal mode of transmission, mtDNA will only provide information on female-mediated gene flow. High levels of female philopatry are therefore often invoked to explain mtDNA patterns that seem unrelated to morphology or nuclear loci (Taberlet and Bouvet 1994; Roca et al. 2005; Caparroz et al. 2009; Urquhart et al. 2009). Analysis of mtDNA gene flow is one way to understand the impact of female demographic history on shaping present population structure within a species (Wilkins and Marlowe 2006).

When previously isolated lineages come into contact, contact zones may be characterized by different degrees of hybridization, introgression, or complete isolation (Ruedi et al. 1997; Wake 1997). Barton and Jones (1983) proposed that cytoplasmic markers (i.e., mitochondria and chloroplasts) are expected to introgress more than nuclear markers, since they are less likely to be linked to genes involved in reproductive,

behavioural, or ecological differences (but see Mishmar et al. 2003; Gershoni et al. 2009). Based on this proposal, mtDNA admixture should be common along the zone of secondary contact between distinct mtDNA lineages. Several intraspecific studies have supported this assumption as different levels of mtDNA admixture were reported at the zones of secondary contact (e.g., Lu et al. 2001; Godinho et al. 2006). Jiggins et al. (1997) proposed that a lack of mtDNA admixture in *Heliconius* butterflies demonstrated a barrier to gene flow. In general, low levels of mtDNA admixture are expected along the zone of secondary contact between diverged populations when gene flow barriers exist.

T. boettgeri appears to show a very pronounced phylogeographical pattern. In this chapter, I aim to identify the areas of secondary contact between the major mtDNA lineages A and B that were described by Gübitz et al. (2005) to allow further investigation of factors that maintain the pattern of mtDNA across Gran Canaria. These lineages seem to have originated through population vicariance caused by volcanic eruptions (Gübitz et al. 2005). Populations are thought to have become isolated by lava flows (Figure 6). Barton and Hewitt (1989) have suggested that contact zones formed by secondary contact should be located in, or move towards, regions of the lowest population density. In this case, it is envisaged that populations will have expanded their ranges into areas previously covered by lava, where secondary contact occurred. Examination of mtDNA lineage distributions reported in Gübitz et al. (2005) broadly indicated where populations belonging to lineage A and B came into contact in the north, northeast and southeast of the island (see Figure 5).

Figure 6. Schematic representation of areas covered by lave flow from volcanic eruptions on Gran Canaria during 5.5 Mya (Gray areas) based on Pérez-Torrado et al. (1995) and Anderson et al. (2009). Possible refugia for *T. boettgeri* during volcanic eruption suggested by Gübitz et al. (2005) are also presented. Broken lines indicate zones of secondary contact between major lineages predicted in this study at north (N), northeastern (NE) and southeastern (SE) of the island.

T. boettgeri are relatively abundant in the south, but are found at lower densities in the central and northern parts of the island, which can be explained by preference for arid low altitude habitats (Brown 1996). It was therefore more difficult to obtain good sample sizes from the northern contact zone and so I concentrated on locating secondary mtDNA contact zones in the south- and northeast of the island.

At any contact zone a cline in character (allele) frequencies must exist (Barton and Hewitt 1985; Barton and Gale 1993; Barton 2000), with the change in allele frequencies being associated with proximity to the centre of the contact zone. The shape of the cline can be determined by the degree of selection against hybrids and the scale of dispersal. Neutral mixing and relatively large dispersal distances will quickly result in a wide shallow cline whereas strong selection against hybrids, coupled with relatively small dispersal distances will result in short, steep clines between populations (Endler 1977; Arnold 1994). MtDNA alone provides no information on hybrids. However, as *T. boettgeri* is expected to be highly philopatric, short, steep clines might be expected.

This chapter aims to obtain insights into the main processes determining geographical structuring of mtDNA across the area of contact by detailed analyses of population substructuring and patterns of mtDNA gene flow. Knowledge of patterns of gene flow is essential to understanding the evolutionary processes acting when lineages are divided in space and time or when formerly divided lineages come into secondary contact (Hewitt 1996). Furthermore, rates of gene flow provide insights into rates of dispersal of an organism, which in turn, help clarify population structure (e.g., García-París et al. 2000).

For species that show female philopatry, male individuals are more likely to migrate across the contact zone (Taberlet et al. 1995). If female philopatry is present in *T. boettgeri*, I would expect to find more males on the "wrong" site of the contact zone than females. However, it is not certain that the observation of individuals from another lineage

on the wrong side of the contact zone is a result of recent migration or interbreeding of populations from different mtDNA lineages. To prevent historical patterns of gene flow from being blurred by present genetic structures, coalescent-based methods have been employed to investigate historical migration among diverged mtDNA lineages within *T. boettgeri*.

Coalescent theory has become useful in population genetic studies, particularly for intraspecific phylogenetics (Posada and Crandall 2001; DeChaine and Martin 2006). Coalescent theory describes the genealogical process within a sample of selectively neutral genes from a population, looking backwards in time (Hudson 1990). The application of coalescent theory to population genetics has provided a statistical framework to test complex hypotheses about historical and current gene flow (Donnelly and Tavaré 1995; Templeton 1998; Fu and Li 1999; Edwards and Beerli 2000). Here, a coalescent-based program, Migrate (Beerli 2008), was employed to identify rates of mtDNA gene flow among populations of *T. boettgeri*. The program combines a conventional maximum-likelihood and coalescent theory based approach with a Metropolis-Hastings Markov chain Monte Carlo (MCMC; Metropolis et al. 1953; Hastings 1970) sampling approach to concentrate the sampling of genealogies in those regions of the genealogy space that contribute most to the final likelihood before the estimated likelihood ratios will be calculated (Beerli and Felsenstein 2001). If *T. boettgeri* lineage A1 and B1 represent two diverged subspecies, it is expected that migration rates should be low between them.

2.2 Methodology

2.2.1 Sampling Regime

All individuals used in this study were obtained from the sample sites shown in Figure 7 (see also Table 1). Sampling regime was determined by preliminary mtDNA

analysis of *T. boettgeri* caught from five sampling sites (JI, JG, M, SA and SD) across the described location of the phylogeographic break (Gübitz et al. 2005). The cytochrome b gene was sequenced for these samples. The sequences were compared to those of Gübitz et al. (2005) and were assigned to the mtDNA lineages described by these authors (detailed in section 2.2.5). After that, the lineages were plotted on a map to determine the approximate mtDNA transition areas. The preliminary study identified the location of the phylogeographic break between lineage A1 and B1 at the village of Juan Grande (Figure 7). Hence, sampling was focused on areas either side of this village toward the edges of adjacent phylogeographic breaks described by Gübitz et al. 2005 (detailed in Figure 5).

In June 2007, intensive sampling was carried out along the south-eastern area, designated the SE transect. Suitable sample sites were selected on the basis of them comprising natural areas with large numbers of rock shelter sites where the geckos are found during the day. The SE transect is approximately 33 km long, covering the area from Arguineguín (Site 02) in the south to Lomo del Cardón (Site 11; Figure 7) in the north. Juan Grande is in the middle of these two extremes. Sample sites were separated by approximately 5 km. Latitudes and longitudes of each site were recorded. Large sample sizes ($n \geq 30$) were obtained wherever possible (see Table 1). Individuals were captured by hand and then, several morphological characters were measured in the field (for more detail, see Chapter 3). Tail tips were excised and preserved in absolute ethanol (99%) for further analysis. Individuals were then released at the exact capture location.

2.2.2 Isolation of genomic DNA

Genomic DNA of *T. boettgeri* was isolated from the tail tips of 425 specimens using the protocol modified from Sambrook et al. (2000). A small amount of tail tip was excised and transferred into a microcentrifuge tube containing 20 µl of 10% SDS, 460 µl Tris-EDTA solution and 10 µl of 20 mg/ml Proteinase K. The mixture was incubated at

56°C overnight. An equal volume of phenol was added and mixed for 15 minutes. The mixture was centrifuged at 14,000 rpm 10 minutes, and the aqueous layer was transferred to a new microcentrifuge tube. The phenol extraction step was repeated by adding an equal volume of phenol, centrifuged at 14,000 rpm, and then the aqueous layer transferred to a new microcentrifuge tube. Next, 1/10 volume of 5M NaCl and 10% CTAB-NaCl were added into the mixture followed by addition of an equal volume of chloroform-isoamyl alcohol (1:25) and mixed for 15 minutes, centrifuged to 5 minutes, and the aqueous upper layer transferred to a new microcentrifuge tube. The chloroform-isoamyl alcohol extraction step was repeated by adding an equal volume of chloroform-isoamyl alcohol, centrifuged at 14,000 rpm for 5 minutes and the aqueous layer transferred to a new microcentrifuge tube. After that, an equal volume of isopropanol was added to the microcentrifuge tube and incubated at room temperature for at least 1 hour before being centrifuged for 15 minutes to pellet the DNA. The pellet was washed with 70% ethanol, and resuspended in 20 µl 0.1XTE buffer pH 8.0. The concentration and quality of genomic DNA of the gecko were observed on 1.4% agarose gel.

2.2.3 Amplification of mtDNA markers using the polymerase chain reaction technique (PCR)

In this study, cytochrome b (cyt b) was chosen as the mtDNA marker because of its high rate of substitution. It is well characterized in geckos, thus, facilitating primer design. In addition, it was previously used in the original study (Gübitz et al. 2005).

Cytochrome *b* (cytb) is the central catalytic subunit of ubiquinal cytochrome *c* reductase (*bc1* complex) in the mitochondrial respiratory chain (Mitchell 1976). It is a transmembrane protein comprising of eight transmembrane helices (Irwin et al. 1991; Esposti et al. 1993) (Figure 8 Inset). Cytb is widely used in phylogenetic studies because it is evolutionarily informative and because of the availability of universal primers (Meyer

1994; Johns and Avise 1998; Berry et al. 2000; Castresana 2001). Amplification of cytb pseudogenes has been reported in several species (Collura and Stewart 1995; DeWoody et al. 1999; Mirol et al. 2000; Lu et al. 2002). Three possible ways to avoid this are i) isolate mtDNA through gradient centrifugation (Tamura and Aotsuka 1988), ii) use RT-PCR (Collura et al. 1996; Triant and DeWoody 2007) and, iii) use species-specific primers with conventional PCR. The first strategy is time consuming and is not appropriate for this research where large sampling sizes are involved. RT-PCR is not suitable due to the difficulty of storage and transporting fresh samples from the field locations. Therefore, species specific primers were designed, and sequences carefully checked for signs of pseudogenes.

PCR primers used in this study are listed in Table 2. Primers were purchased from MWG (Germany). In order to obtain several *T. boettgeri* cytb sequences which were of sufficient length to allow design of new primers, PCR was first performed with primers MTA-s and MTF-s (see Table 2). Consequently, the PCR sequences were aligned to locate the conserved regions (see section 2.2.4), and five *T. boettgeri*-specific cytb primers were designed based on those consecutive regions and applied to all *T. boettgeri* specimens.

For amplification of mitochondrial markers, the reaction was performed in 25 µl by using 100 ng of genomic DNA, 1X Premix PCR reaction buffer (75 mM Tris-HCl pH 9.0, 50 mM KCl, 20 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$ and 0.25 units of DNA polymerase) (Sigma Aldrich) and 0.4 pmole of each primers. Forty cycles of PCR were carried out after an initial denaturation step at 94°C for 5 minutes. Each cycle consisted of three holding temperatures, 94°C for 1 minute to denature DNA, 1 minute at optimized temperature for annealing (see Table 2) and 72°C for 2 minutes for extension, followed by a final extension at 72°C for 10 minutes.

Table 1 Locations and number of *Tarentola boettgeri* used in this study.

Site	Local Name	Geographical location	Number of individuals
01	Juan Grande	27° 49.086'N 15° 27.628'W	31
02	Arguineguín	27° 45.530'N 15° 40.374'W	30
03	Barranco de Fataga (near Maspalomas)	27° 46.725'N 15° 34.988'W	30
04	Carrizal	27° 53.938'N 15° 24.655'W	31
05	Crude de Arinaga	27° 53.155'N 15° 26.385'W	31
06	Sardina del Sur	27° 51.223'N 15° 28.096'W	30
07	Barranco del Draguillo (Planta de Residuos Sólidos)	27° 48.666'N 15° 28.913'W	32
08	Mesa de las Canãdas	27° 47.440'N 15° 30.116'W	30
09	San Agustín	27° 46.563'N 15° 32.042'W	30
10	Llanos de Arinaga	27° 51.422'N 15° 25.118'W	30
11	Lomo del Cardón	27° 55.386'N 15° 23.962'W	31
12	Tablero	27° 46.789'N 15° 37.385'W	30
13	Playa del Inglés	27° 45.943'N 15° 34.194'W	17
14	Castillo del Romeral	27° 48.021'N 15° 28.178'W	15
JI	Jinámar	Unspecified	3
JG	Juan Grande	Unspecified	6
M	Malfu	Unspecified	3
SA	San Agustin	Unspecified	1
SD	Sardina del Norte	Unspecified	1
Tb	Pinor de Tamadb, Artenara	Unspecified	1
AC	Alcampo	Unspecified	4
LS	Las Salinetas	Unspecified	3
OG	Ojos de Garza	Unspecified	5
EC	El Cortijo	Unspecified	1

Note: Samples from Site Tb, AC, LS, OG and EC were only used for reconstruction of the phylogenetic tree.

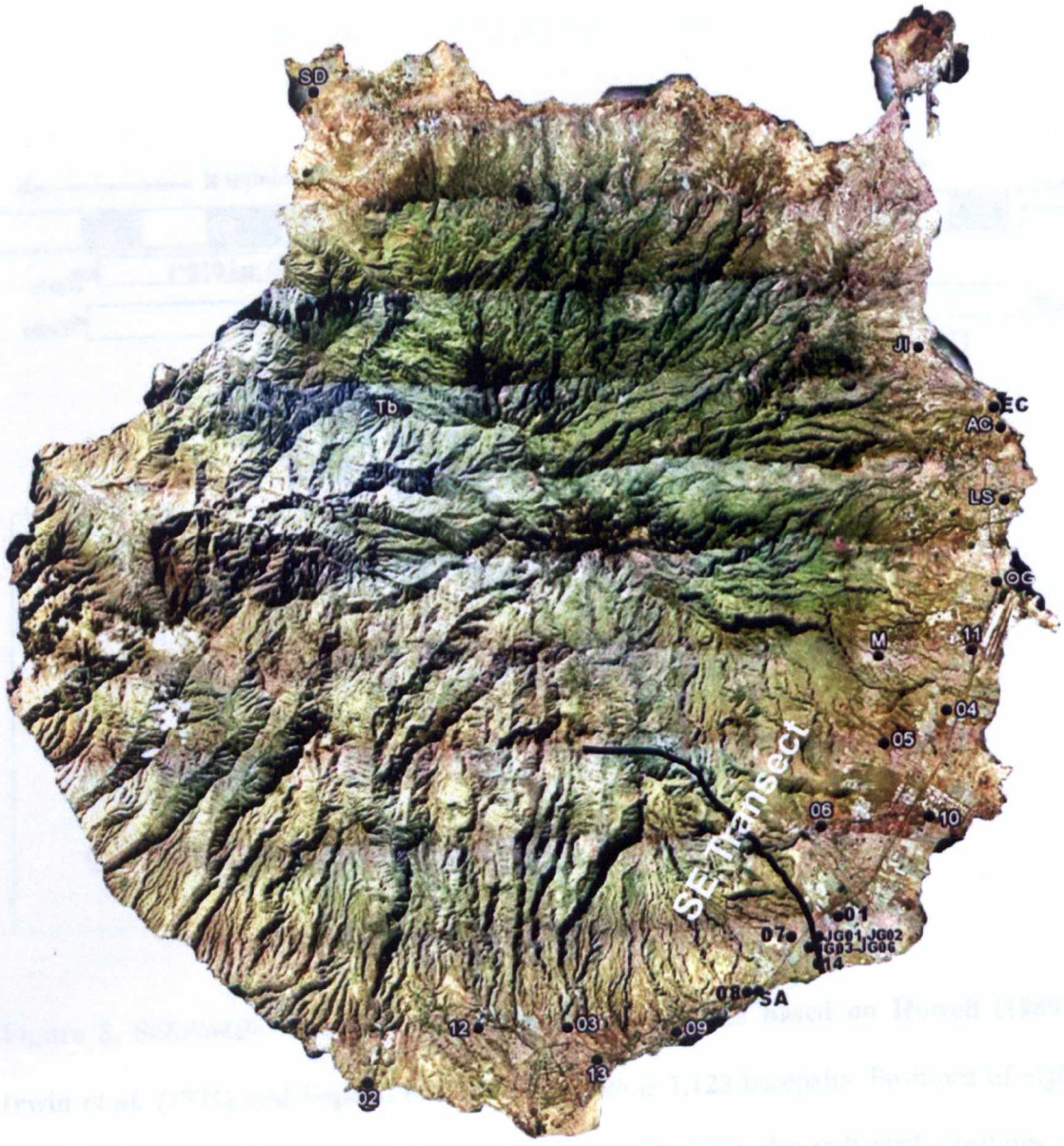


Figure 7. Geographical map of Gran Canaria with all sampling sites (indicated using dots). Names given to each site correspond to information in Table 1. The centre of the transitional zone between lineage A1 and B1 (identified in this study) is shown as a line. (The satellite image was created using Google Earth version 5.2.)

Figure 8. Schematic representation of the cytb sequence based on Howell (1989), Irwin et al. (1991) and Esposti et al. (1993). *Cytb* is 1,122 basepairs. Positions of eight helices are indicated as closed boxes. N- and C- terminus are also indicated. Positions of primers used in Gübitz et al. (2005) and this study are indicated as arrow. Picture drawn to scale (based on cytb sequence of *T. mauritanica* EU443255: Albert et al. 2009). Inset shows secondary structure diagram of cytb and its position with respect to the inner membrane of mitochondria. The filled squares represent heme groups. (Modified from Esposti et al. (1993)).

Table 2 Oligonucleotide used in screening of mtDNA markers and their descriptions.

Primer	T_m (°C)	Sequences of primer from 5' to 3'	References	Expected PCR product
MTA-s		ATCTCAGCATGATGAAACTTCG	*Anolis sp.* cytochrome b (Thorpe et al. 2008)	1100 bp
MTF-s		TTTGGTTTACAAGACCAATG		
CBT-F1	50.6	CCATGAGGACAAATATCATTCTG	This study	See Figure 8
CBT-F2	66.0	CTGAGGCGCAACCGTAATCACCACC		
CBT-R1	49.7	GTAAATGGGGGTTCTACTTGG		
CBT-R2	65.1	TAGGCGACTGAGGCGGCCTGG		
CBT-R3	59.4	TACTGGTTGGCCGCCGCTTC		

The PCR products were characterized on 1.4% agarose gel containing 0.5 mg/ml of Ethidium Bromide (EtBr) and observed under the UV light. In order to remove unincorporated nucleotide and primers, the PCR product was purified using QIA quick PCR purification kit (QIAGEN) as manufacturer recommendation.

The ABI PRISM™ Big Dye Terminator Sequencing Kit (Applied Biosystems) provided all of the solutions and reagents required for the sequencing reaction. The reaction was performed in 20 µl by using 200 ng of purified PCR product, 1 pmole of sequence-specific primer, 4 µl Premix (ABI PRISM™) and 0.5X sequencing buffer. Nucleotide sequences were carried out by Source Biosciences, Ltd.

2.2.4 DNA sequence analysis

Sequence comparisons were carried out using the BLAST program (Altschul et al. 1997) within GenBank. The sequence alignment programs, BIOEDIT (Hall 1999) and MEGA version 4 (Tamura et al. 2007) were used to align and correct the results. The mean pairwise distances and standard error (based on 500 bootstrap replicates) between and

within lineages were calculated, using p-distances for nucleotide and amino acid (Nei and Kumar 2000).

The cytb sequences were translated into amino acid sequences and checked for signs of pseudogene contamination (e.g., frameshift mutations and stop codons) as described in Bensasson et al. (2001). Haplotype assignment was performed using TCS program version 1.21 (Clement et al. 2000).

2.2.5 Phylogenetic tree reconstruction

The cytb sequences amplified here partially overlapped with those of Gübitz et al. (2005) (see Figure 8). Thus, thirteen 1.1 kb cytb sequences that I obtained for primer design were aligned with those of Gübitz et al. (2005) (AY841905.1- AY841944.1), and a cladogram was constructed to allow comparison of lineages between the two studies (see Appendix 1).

The hierarchical likelihood ratio test implemented in MODELTEST version 3.7 (Posada and Crandall 1998) was used to evaluate the most appropriate substitution models for phylogenetic trees using the standard Akaike information criterion. MODELTEST supported the GTR+G model with estimates of the gamma distribution shape parameter of 0.6998 and 1.3187 for codon partitions 1 and 2, respectively, and the F81+G model with gamma shape parameter estimate of 0.7373 for position 3.

MrBayes software version 3.1.2 (Ronquist and Huelsenbeck 2003) was used to obtain the mtDNA phylogeny by Bayesian inference. The Bayesian approach provides a consensus tree from the posterior distribution of trees, with node support assessed by posterior probabilities (Huelsenbeck et al. 2001). Two independent analyses were run, each with four Markov Chain Monte Carlo (MCMC) chains. Chains were run for $2x10^6$ generations, and were sampled every 100 generations. The chain lengths and convergence were determined by the standard deviation (SD) of split frequencies. As suggested in the

MrBayes v3.1 Manual, the runs are likely to have converged on the same posterior when the SD drops below 0.01. Once the chain had converged, a 50% majority-rule consensus tree was created from the post-burn-in samples comprising 15,001 trees.

2.2.6 Testing population substructure

The genetic structure of *T. boettgeri* was investigated by analysis of molecular variance (AMOVA) as implemented in Arlequin program version 3.5 (Excoffier and Lischer 2010). AMOVA is a method of estimating population differentiation directly from gene frequencies and testing hypotheses about such differentiation (Cockerham 1969, 1973; Excoffier et al. 1992). It uses the fixation index to analyze the differentiation among the populations from different groups (or sampling locations). Hypotheses are tested using the null distribution of the variance components. If the variance of subpopulations does not significantly differ from the null distribution of the variance of the population, the hypothesis that those subpopulations are differentiated from the larger population is rejected (Excoffier et al. 1992; Excoffier 2001). The significance of the covariance components associated with the different possible levels of genetic structure (within populations, within group of populations, among groups) is tested using non-parametric permutation procedures (Excoffier et al. 1992).

Genealogical relationships within lineage A and B were also represented using the Median Joining (MJ) method (Bandelt et al. 1999) as implemented in Network version 4.6 (Fluxus Technology Ltd.). MJ is used for constructing networks from recombinant-free population data based on a parsimony criterion (Bandelt et al. 1999).

2.2.7 Studying pattern of mtDNA gene flow across the SE transect

The program MIGRATE version 3.1.6 (Beerli 2008) was used to estimate long-term gene flow between mtDNA lineages A1 and B1. I chose to estimate historical migration rates (μ) and mutational-scale effective population sizes (θ) using the maximum

likelihood coalescent approach (Beerli and Felsenstein 1999; 2001). (Note that μ is the mutation scaled migration rate.) The search strategy used 10 short-chains and 2 long-chains. The program was run twice. Following a burn-in of 10^4 iterations, the first run was carried out with 10^5 and 10^6 genealogies for short- and long chains, respectively, with a sampling interval of 20 generations. The second run sampled 10^6 and 10^7 genealogies using short- and long chains, with a sampling interval of 100 generations. Migration rates (μ) between each pair were plotted against geographical distance between each site. To enable a comparison of results between mtDNA and nuclear data, numbers of migrants per generation were obtained from the product of the migration rate (μ) and θ, as was suggested in the user manual.

2.3 Results

2.3.1 Cytb sequence variation

A total of 389 high quality cytb sequences were obtained (which corresponded to 176 unique haplotypes; see Appendix 2). Comparison of these sequences with those in GENBANK confirmed sequence identity with *T. boettgeri* cytb sequences submitted by Carranza et al. (2000) and Gübitz et al. (2005). Translation to amino acid sequences did not reveal evidence of pseudogene amplification. Regions containing ambiguous bases (at the beginning and end of the sequences) were removed providing 608 bp for analysis.

The 608 bp were aligned and no indels were detected (see Figure 9). The mean base composition was 0.30 (A); 0.26 (T); 0.32 (C) and 0.13 (G), and the transition/transversion ratio was 1.84 (outgroup included: *T. delalandii*). Numbers of nucleotide and amino acid segregating sites (including outgroup) are 40.15% and 5.30%, respectively.

2.3.2 Phylogenetic relationships within T. boettgeri and location of area of secondary contact

The tree based on the 176 haplotypes contained five major well-supported branches consistent with Gübitz et al. (2005) (Figure 10). Bayesian posterior probabilities strongly supported major clades (1.00), except for Clades A1 and A2 (0.79). Clades A and B are reciprocally monophyletic. Relationship within lineage A are clear: lineage A1 and A2 are more closely related, while A3 outgroups these two lineages. Within Clade A1 and B1, there are also two well-supported branches (1.00) which I designate Subclade A1a, A1b, B1a and B1b.

Concordance between this tree and that of Gübitz et al. (2005) allowed all haplotypes to be assigned to clades and subclades without ambiguity (see Appendix 2). Average uncorrected nucleotide (*p*) distance (Nei and Kumar 2000) were calculated. The uncorrected raw divergence between Clade A and B is 12.0%, which is higher than previously reported (10.9%; Gübitz et al. 2005). Similar to previous studies, divergence within Clade A (10%) is higher than within Clade B (4.9%). The uncorrected raw divergence within Subclades (see Table 3) indicated that divergence within Subclades A1a and A1b is higher than between Subclades B1a and B1b.

Table 3 Average uncorrected nucleotide p-distances within and among mtDNA subclades A1 and B1.

Clade	A1a	A1b	B1a
A1b	0.023		
B1a	0.122	0.121	
B1b	0.123	0.123	0.014

Figure 9. Schematic representation of nucleotide consensus sequence alignment of *T. boettgeri* with *T. delalandii* as an outgroup. Asterisks indicated base positions, which varied between lineage A and B. Lines indicated position of helices D – H (Esposti et al. 1993).

Figure 9 (cont.)

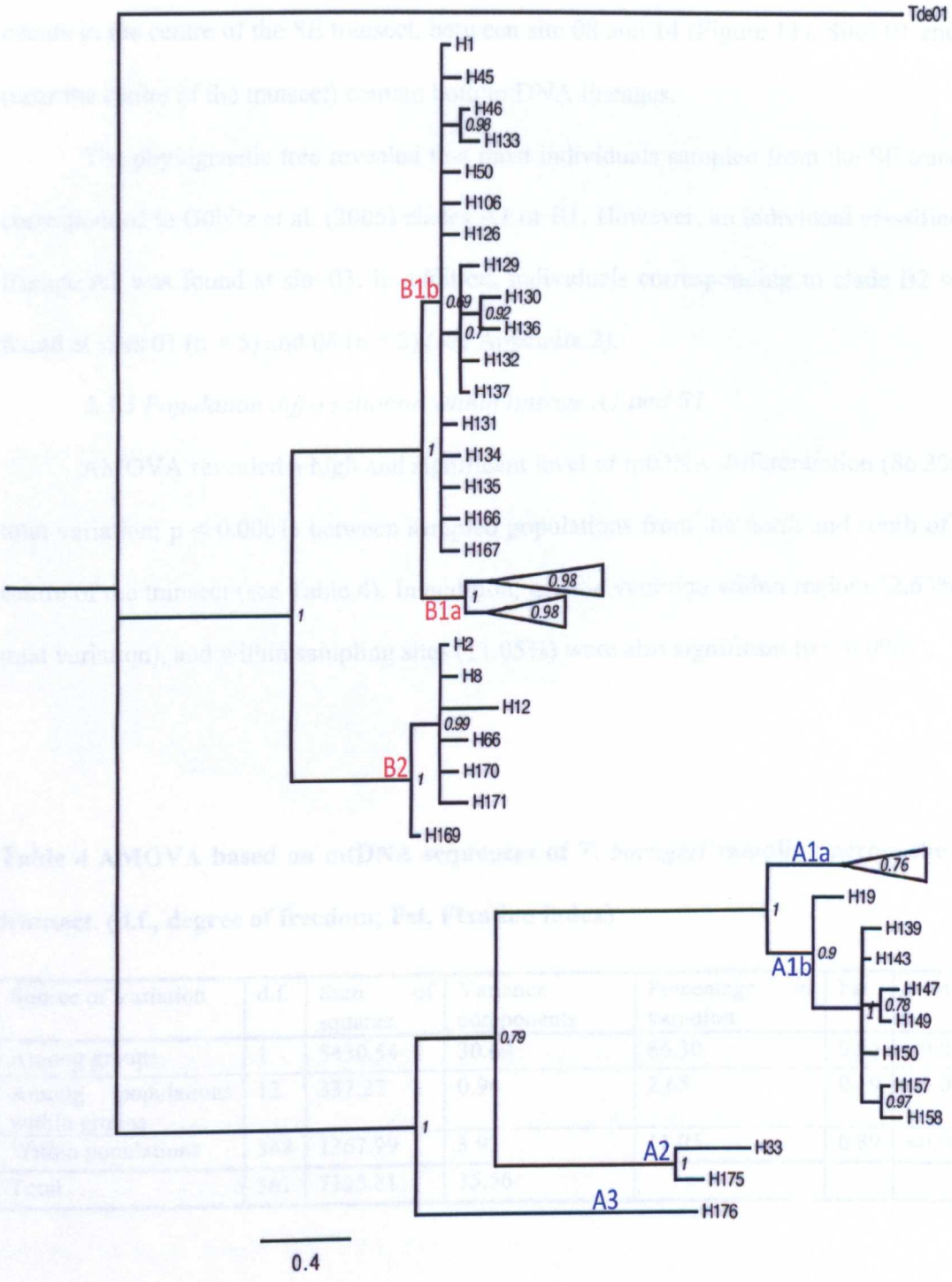


Figure 10. Bayesian Inference tree constructed from 176 *cyt b* haplotypes of *T. boettgeri* across the island with *T. delalandii* (Tde01) as an outgroup. Bayesian posterior support values are indicated at each node. This analysis shows the posterior support for the main clades to be discussed. Detailed analyses of relationships within these clades are shown in Figures 12 and 13.

The main phylogeographic break between populations with A1 and B1 haplotypes occurs in the centre of the SE transect, between site 08 and 14 (Figure 11). Sites 01 and 07 (near the centre of the transect) contain both mtDNA lineages.

The phylogenetic tree revealed that most individuals sampled from the SE transect corresponded to Gübitz et al. (2005) clades A1 or B1. However, an individual classified as lineage A2 was found at site 03. In addition, individuals corresponding to clade B2 were found at sites 01 (n = 5) and 06 (n = 2) (see Appendix 2).

2.3.3 Population differentiation within lineage A1 and B1

AMOVA revealed a high and significant level of mtDNA differentiation (86.3% of total variation; $p < 0.0001$) between sampled populations from the north and south of the centre of the transect (see Table 4). In addition, genetic variation within regions (2.65% of total variation), and within sampling sites (11.05%) were also significant ($p < 0.0001$).

Table 4 AMOVA based on mtDNA sequences of *T. boettgeri* sampling across the SE transect. (d.f., degree of freedom; Fst, Fixation index)

Source of Variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fst	p-value
Among groups	1	5450.54	30.69	86.30	0.86	<0.0001
Among populations within groups	12	337.27	0.94	2.65	0.19	<0.0001
Within populations	348	1367.99	3.93	11.05	0.89	<0.0001
Total	361	7155.81	35.56			

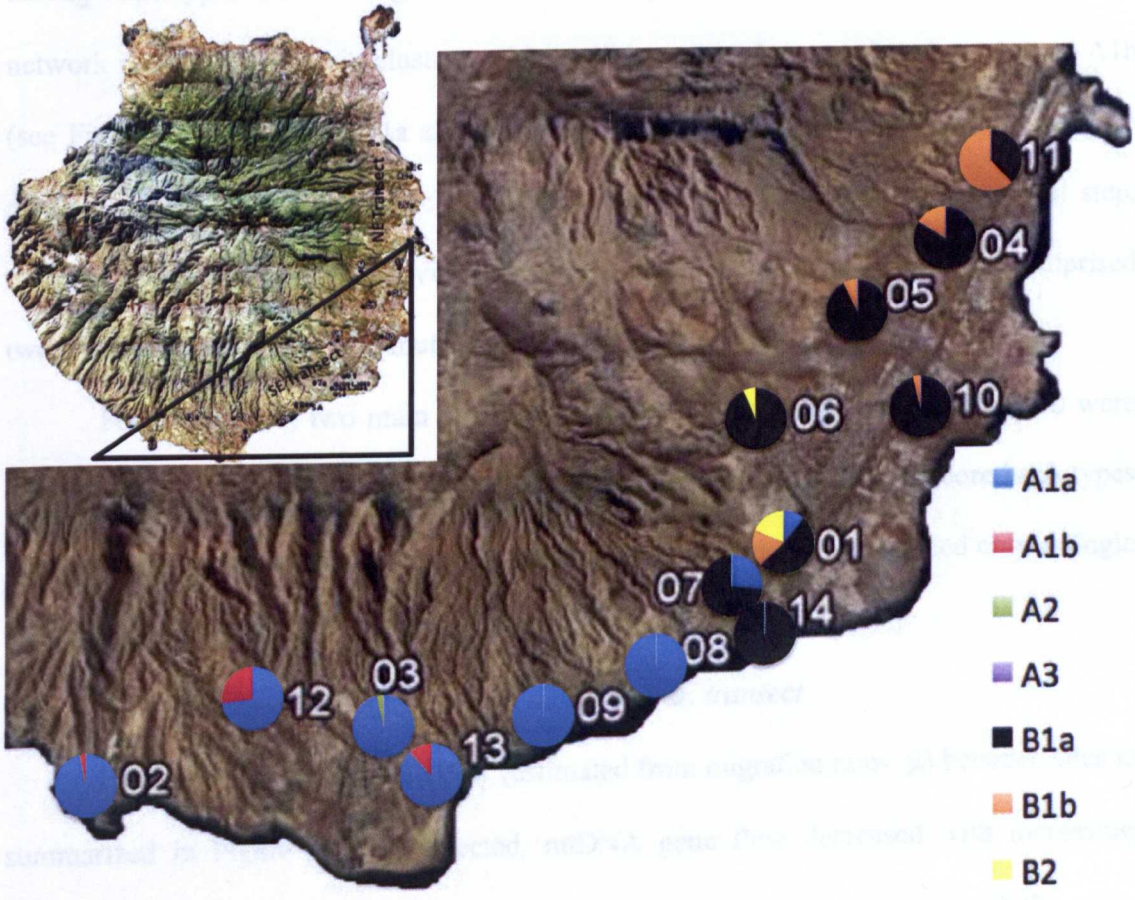


Figure 11. Geographical map of the SE transect. Pie charts show clade membership frequencies at the different sampling sites. Individuals from Clades A1 and B1 are separated into Subclades A1a/A1b and B1a/B1b, respectively. Inset: Map of Gran Canaria with the SE transect indicated within the triangle.

The median-joining (MJ) networks provided further resolution of the relationship among haplotypes from lineages A1 and B1 (Figure 12 and 13). For lineage A1, the network identified two main clusters, which will be referred to as subclades A1a and A1b (see Figure 12). Subclades A1a and A1b were connected by seven substitutions and one median vector. Within each subclade, most haplotypes differed by one mutational step, with a few exceptions. Most individuals were grouped into subclade A1a, which comprised two clusters connected by two mutational steps (Figure 12B).

For lineage B1, two main clusters corresponding to subclades B1a and B1b were connected by four substitutions (Figure 13). Subclade B1a comprised two core haplotypes connected by two substitutions and a median vector. Subclade B1b contained only a single cluster of haplotypes.

2.3.4 Pattern of mtDNA gene flow across the SE transect

Patterns of long-term gene flow (estimated from migration rates: μ) between sites is summarized in Figure 14. As expected, mtDNA gene flow decreased with increasing distance between sites. Migration rates are particularly high between sites with the same mitochondrial clades (Figure 14, see also Table 5). However, migration rates are lower between sites with different mtDNA clades. The results also indicated substantial gene flow between various sites containing either mtDNA lineage A1 or B1 and sites 01 and 07, where both lineages co-occurred. They also strongly suggested asymmetric gene flow from lineage A1 to B1 as migration from A1 to B1 occurred at the higher rates than from B1 to A1 (Table 5).

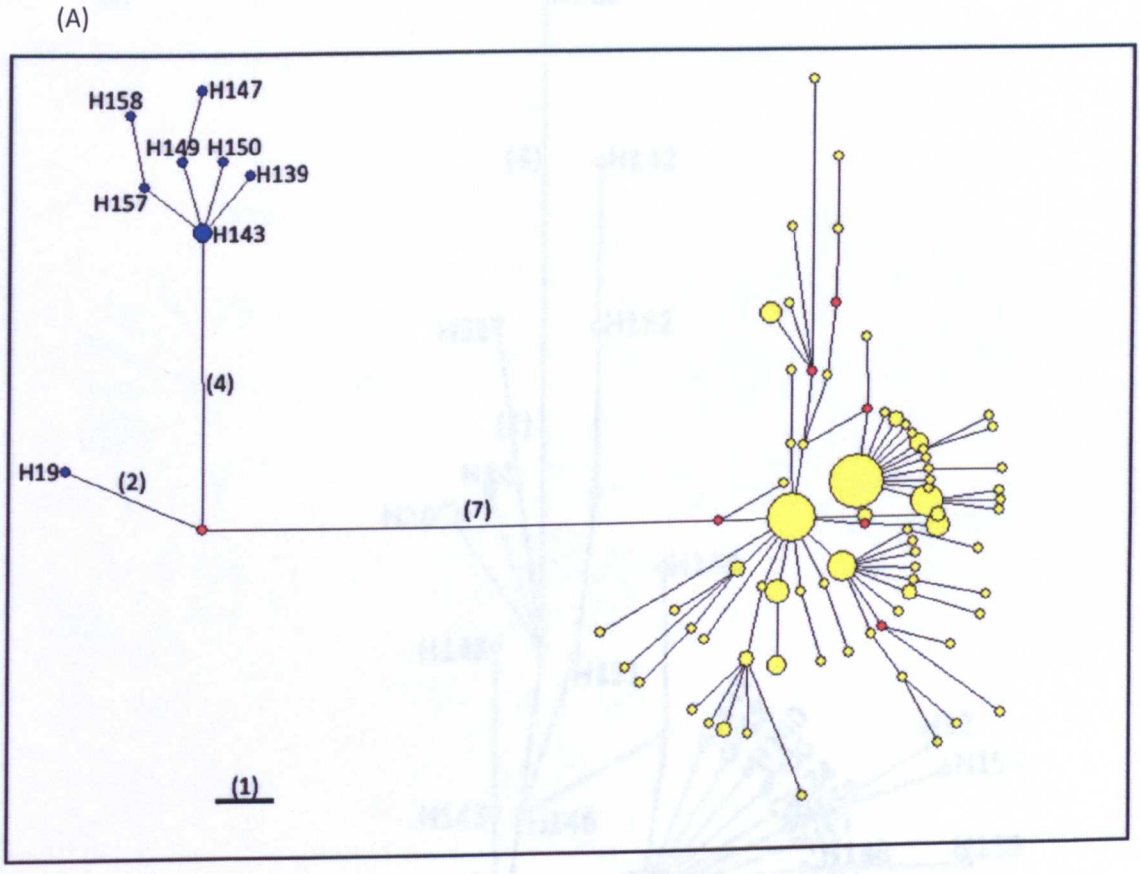


Figure 12. (A) A median-joining (MJ) network based on mtDNA sequences from lineage A1. Haplotypes are represented by circles, with circle size proportional to haplotype frequencies. Yellow circles represent the haplotypes belonging to lineage A1a, while lineage A1b are blue circles. Median vectors (red circles) are also presented. The lengths of the lines are proportional to the number of substitutions (shown in brackets). The scale of a single substitution is shown in the lower left corner. **(B) Fine scale of MJ network for lineage A1a.**

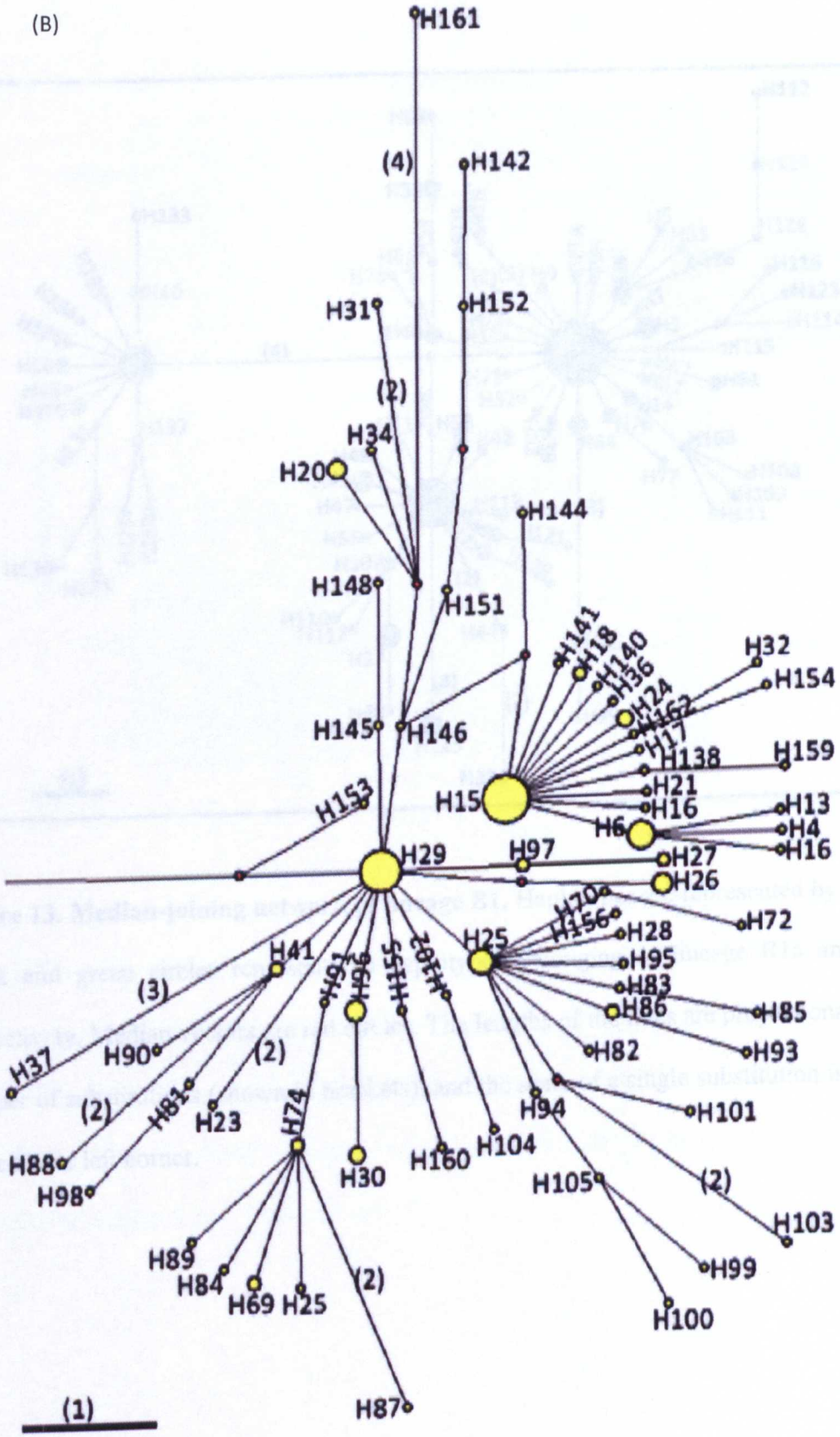


Figure 12 (cont.)

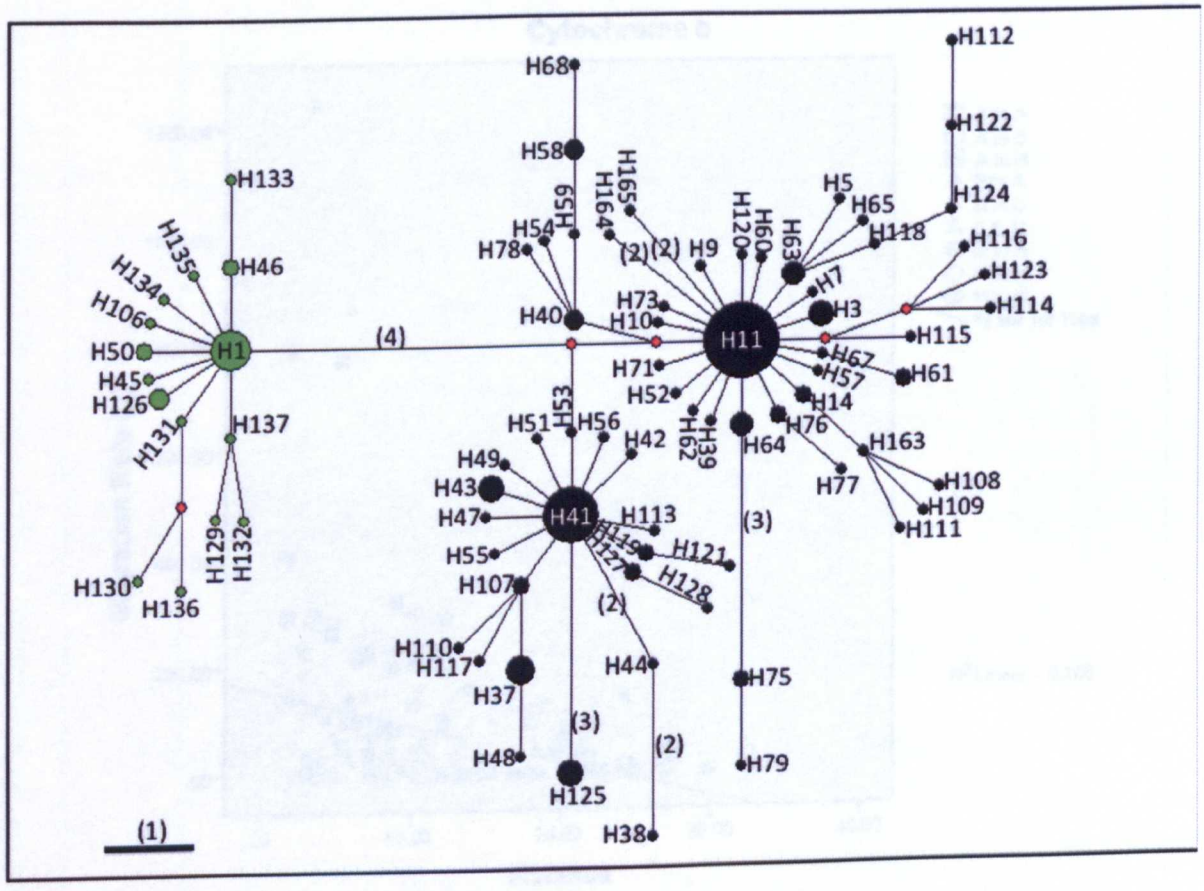


Figure 13. Median-joining network of lineage B1. Haplotypes are represented by circles. Black and green circles represent the haplotypes belonging to lineage B1a and B1b, respectively. Median vectors are red circles. The lengths of the lines are proportional to the number of substitutions (shown in brackets), and the scale of a single substitution is shown in the lower left corner.

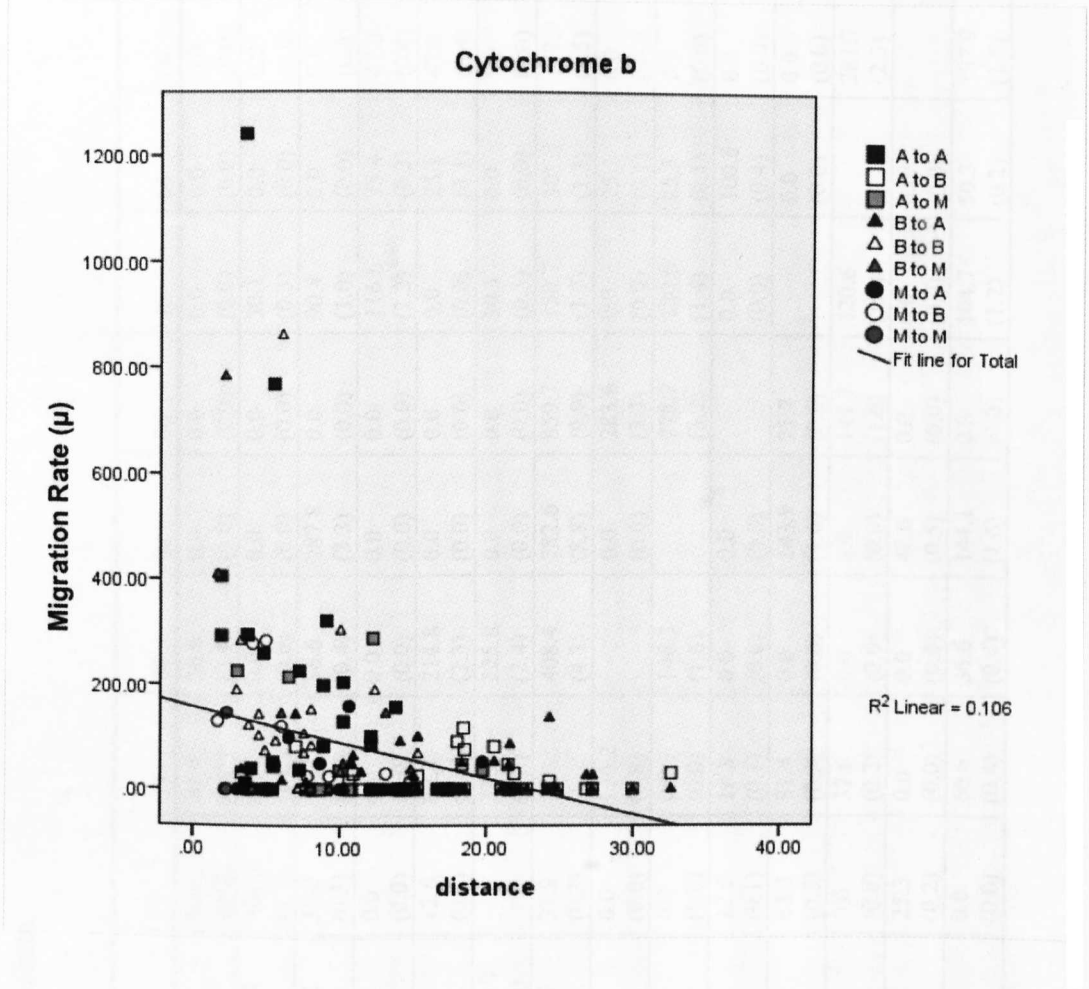


Figure 14 Scatter plots of migration rates (μ) and geographical distances (km) between sampling sites. Squares represent migration rates from sites comprising pure mitochondrial clade A. Triangles represent migration rates from sites composed of mitochondrial clade B. Circles represent migration rates from two localities that contained both mtDNA clades A and B (site 01 and 07).

Table 5 Estimates of mtDNA migration rates between sites across the SE transect. Sites are arranged according to their position on the transect. Numbers of migrants per generation are in brackets.

To site / From site	02	12	13	03	09	08	14	07	01	06	10	05	04	11
02		0.0 (0.0)	204.5 (1.6)	320.6 (4.1)	0.0 (0.0)	0.0 (0.0)	81.4 (0.5)	36.6 (0.4)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	30.1 (0.2)
12	52.1 (0.3)		767.6 (5.9)	0.0 (0.0)	198.9 (2.1)	101.2 (0.7)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	30.1 (0.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
13	129.2 (0.8)	44.1 (0.5)		292.4 (3.7)	294.2 (3.2)	38.0 (0.3)	16.3 (0.1)	36.6 (0.4)	287.8 (3.3)	0.0 (0.0)	90.4 (1.0)	0.0 (0.0)	0.0 (0.0)	15.1 (0.1)
03	0.0 (0.0)	40.4 (0.4)	404.8 (3.1)		0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	116.6 (1.3)	75.4 (0.3)	47.0 (0.4)	0.0 (0.0)
09	156.4 (1.0)	82.6 (0.9)	0.0 (0.0)	259.9 (3.3)		12.6 (0.1)	82.4 (0.5)	214.8 (2.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	25.1 (0.1)	47.0 (0.4)	0.0 (0.0)
08	0.0 (0.0)	84.5 (0.9)	225.8 (1.7)	0.0 (0.0)	1240.0 (13.4)		32.6 (0.2)	225.8 (2.4)	0.0 (0.0)	0.0 (0.0)	30.1 (0.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
14	52.1 (0.3)	0.0 (0.0)	47.9 (0.4)	32.5 (0.4)	142.7 (1.5)	37.9 (0.3)		408.4 (4.3)	782.6 (8.8)	859.7 (9.9)	150.7 (1.7)	301.8 (1.3)	187.9 (1.5)	0.0 (0.0)
07	52.1 (0.3)	0.0 (0.0)	0.0 (0.0)	158.2 (2.0)	99.3 (1.1)	0.0 (0.0)	130.2 (0.8)		0.0 (0.0)	283.6 (3.3)	0.0 (0.0)	25.1 (0.1)	0.0 (0.0)	15.1 (0.1)
01	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	49.6 (0.5)	0.0 (0.0)	0.0 (0.0)	146.3 (1.6)		278.2 (3.2)	120.6 (1.4)	25.3 (0.1)	0.0 (0.0)	30.2 (0.2)
06	0.0 (0.0)	0.0 (0.0)	90.3 (0.7)	0.0 (0.0)	49.6 (0.5)	12.6 (0.1)	16.3 (0.1)	0.0 (0.0)	0.0 (0.0)		0.0 (0.0)	100.6 (0.4)	0.0 (0.0)	0.0 (0.0)
10	26.1 (0.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	63.2 (0.5)	81.4 (0.5)	0.0 (0.0)	143.9 (1.6)	71.7 (0.8)		0.0 (0.0)	0.0 (0.0)	67.4 (0.5)
05	26.1 (0.2)	84.5 (0.9)	0.0 (0.0)	0.0 (0.0)	99.3 (1.1)	0.0 (0.0)	32.6 (0.2)	0.0 (0.0)	0.0 (0.0)	141.7 (1.6)	120.6 (1.4)		281.7 (2.3)	90.4 (0.7)
04	0.0 (0.0)	0.0 (0.0)	45.1 (0.3)	0.0 (0.0)	49.6 (0.5)	25.3 (0.2)	0.0 (0.0)	0.0 (0.0)	48.0 (0.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)		0.0 (0.0)
11	0.0 (0.0)	0.0 (0.0)	135.4 (1.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	66.6 (0.4)	36.6 (0.4)	144.1 (1.6)	0.0 (0.0)	104.7 (1.2)	50.3 (0.2)	187.9 (1.5)	

2.4 Discussion

2.4.1 Cytb sequence variation and mtDNA lineages distribution across the SE transect

Sequence divergence was found to be greater than that reported by Gübitz et al. (2005). The main reason for this appears to be the different location of the primers on the cytb gene. Primers designed in this study are located at the C-terminus of cytb (see Figure 8) while primers used by Gübitz et al. (2005) were located at the N-terminus (Kocher et al. 1989; Moritz et al. 1992). It is generally known that the C-terminus of cytb has a higher substitution rate than the N-terminus (Howell 1989; Edwards et al. 1991). In addition, cytb sequences used in this study included helices E and H, which are the least conserved regions (Esposti et al. 1993). This is because they do not contain heme-binding site, and, are thus less involved in the electron transfer chain (Irwin et al. 1991; Zhang et al. 1998).

This study confirmed the five major mtDNA lineages of *T. boettgeri* determined by Gübitz et al. (2005). The results were also geographically concordant with the previous study: mtDNA lineage A1 is distributed along the southern coast of Gran Canaria, while lineage B1 is found in the south-east of the island. However, the findings differ significantly from Gübitz et al. (2005).

First, Gübitz et al. (2005) reported no mtDNA admixture between lineage A1 and B1. This would be unusual since it is typical to find mtDNA admixture at the area of secondary contact (e.g., Tomaru et al. 1998; Vallianatos et al. 2001; Phillips et al. 2004; Gum et al. 2005; Pinceel et al. 2005; Kuchta 2007). However, it is likely to be due to Gübitz et al.'s (2005) less intensive sampling across this area. The more intensive sampling design used here allowed identification of mtDNA admixture at Site 01 and 07, where lineages A1 and B1 co-exist. Nevertheless, the fact that it occurred at only two sites,

despite short distances between sites and large sample sizes, provides strong evidence that the degree of mtDNA admixture is surprisingly limited.

Second, the finding of haplotypes corresponding to clade A2 and B2 in the SE transect also contradicts Gübitz et al. (2005). These authors showed that lineage A2 is restricted to the north-western and B2 to the north-eastern parts of the island. The geographical ranges of lineage B1 and B2 meet in the east of the island. It is possible that low levels of southwards migration of mtDNA lineage B2 may explain this. However, if this was the case, lineage B2 should be observed along the SE transect. Instead it is found at only two sites (site 01 and 06) near the centre of the transect, suggesting that this is unlikely. This study does not exclude the hypothesis that both lineages may have extended their ranges via other areas rather than along the coast. However, these areas are beyond the SE transect and Gübitz et al.'s (2005) description is insufficient to examine this hypothesis. Thus, it remains unclear whether the observation of these northern lineages in the south of the island are solely a result of natural expansion.

Another possibility was that individuals were introduced from north to the south-east of the island. The effects of human activities on endemic species by alteration of the environment and introduction of species into new habitats are well-known (see review in Vitousek et al. 1997). The impacts of human activities on the Canary islands fauna have also been reported in insects (De La Rúa et al. 2001; Vargus et al. 2004), birds (Suárez et al. 2012), amphibians (Recuero et al. 2007), and reptiles (Cejudo and Márquez 2001; Morales et al. 2009). In Gran Canaria, human activities were shown to be responsible for within-island genetic diversity of the honeybee, *Apis melifera* (De La Rúa et al. 2001). In addition, studies of colonization and range expansions of *Hemidactylus* geckos in Mediterranean and Atlantic islands, including Gran Canaria, indicated that they were the result of human-mediated transportation (Carranza and Arnold 2006). Unlike *Hemidactylus*

geckos and *A. melifera*, there was no evidence suggesting that *T. boettgeri* has a commensal existence with humans. Nevertheless, genetics and biogeographical studies of an amphibian, *Hyla meridionalis*, across the Canary islands and within each island revealed the patterns associated with movements among high activities areas, such as cultivations and livestocks areas (Recuero et al. 2007). I cannot rule out this possibility for *T. boettgeri*, especially as sites 01, 03 and 06 were located in the the villages, next to the main roads (pers. obs.). As the geckos and their eggs are small, it remains possible that the individuals from the northern clades may have been accidentally transported to these southern sites. Thus, human introduction may be the best explanation for the occurrences of lineages A2 and B2 at these locations (particularly the unexpected presence of a single individual from lineage A2 at site 03).

2.4.2 MtDNA gene flow across the zone of secondary contact

Clines in haplotype frequencies indicated that mtDNA lineages A1 and B1 meet at a narrow contact zone (approximately 3.3 km wide). This zone is situated at equal distances from the two putative refugia (Gübitz et al. 2005) from where they originated. In Gübitz et al. (2005), putative refugia were proposed based on areas unaffected by lava flow during two recent volcanic eruptions (see Figure 6). However, due to a high rate of erosion in the south of the island, it is difficult to predict the actual scale of lava flow (Mehl and Schminke 1999). Therefore, in this study the refuge hypothesis (originally proposed by Endler 1982) and geology of the island were used for evaluation of the refugia. In agreement with the refuge hypothesis (Endler 1982) which predicts that secondary contact zones will be equidistant from refugia, the location of refugia should be within the areas proposed by Gübitz et al. (2005). In addition, there is evidence to suggest that mountain ridge environments remained unaffected by the lava flows (e.g., Anderson et al. 2009). Thus, it is possible that high altitude habitats, particularly Fataga and east of Santa Lucia

de Tirajana were the refugia for lineages A1 and B1, respectively. This seems to provide additional support for Gübitz et al's hypothesis of the geographical origins of lineages A1 and B1.

Interestingly, the SE contact zone is located at the exact location of a reported phylogeographic break between mtDNA lineages of *C. sexlineatus* (Pestano and Brown 1999). It is not unusual for phylogeographic breaks within species to cluster in particular geographic locations, which is often explained by species range expansion from their refugia (e.g., Avise 1992; Hewitt 1996; Guillaume et al. 2000; Hewitt 2001; Swenson and Howard 2005). Quite often, the mtDNA lineages appear to have diverged at different times (as evidenced by different levels of sequence divergence for the same genes) but show concordant geographic patterns (Guillaume et al. 2000; Hewitt 2000). This is the case with *C. sexlineatus* which shows a much shallower divergence that appears to have arisen during the last period of within-island volcanism and so postdates sequence divergence of major mtDNA clades in *T. boettgeri* around 2 Ma. MtDNA data on the third lizard species on Gran Canaria, *Gallotia stehlini* are unpublished but apparently show a very shallow and geographically very distinct pattern (R. Brown, pers. comm.).

Given the approximate age of the common mtDNA ancestor of these two clades, and the scenario proposed by Gübitz et al. (2005), the time since secondary contact would be expected to be quite ancient, not recent. High levels of mtDNA admixture are expected among populations with no barriers to gene flow (Comas et al. 2004; Gum et al. 2005). Although it is not possible to estimate the time when secondary contact between lineage A1 and B1 took place from this study, it should have occurred sometime after the last period of volcanic activity (between approximately 3 Mya and present). We detected very limited historical mitochondrial gene flow across this region, which is clearly indicative of a barrier. There is no evidence of a present-day physical barrier and there is no evidence of

current or recent geological events that would have interrupted genetic exchange between populations in this region. One possible hypothesis is that there is a biological barrier to mtDNA gene flow between populations, as could occur due to genetic incompatibility between nuclear and mtDNA genomes (Dasmahapatra et al. 2002; Kim and Sappington 2004). This will be explored in chapter 5.

The stability and maintenance of phylogeographic patterns depends on the longevity and vagility of the organisms (Hewitt 1996). Thus, organisms with low vagility should exhibit low dispersal rates and show strong phylogeographic patterns (Branch et al. 2003; Martínez-Solano et al. 2007). There are no reports on the life span of *T. boettgeri*. However, inferred mtDNA migration rates between populations containing the same clades and separated by <5 km appear relatively high, but generally decline rapidly with increasing geographical distance. The width of the contact zone is also related to dispersal (Endler 1977; Barton and Hewitt 1985) with the narrow SE contact zone indicating low dispersal. Together these results suggest that low vagility or high philopatry may help explain the geographical patterns. Nevertheless it seems unlikely that this has a major influence on maintaining the mtDNA structure across the centre of the transect. The number of female (7) and male (4) A1 individuals observed on the "B1 side" of the contact zone does not correspond to what should be expected from species with strong female philopatry. Thus, it implies intrinsic factors, such as cyto-nuclear incompatibility, may have more influence on maintaining the contact zone than female philopatry.

2.4.3 Population substructure within mtDNA lineages of T. boettgeri

An important finding of my study is the detection of quite deep lineages within lineages A1 and B1 (see Table 3). This allowed further analysis of mtDNA structuring in this geographical region. The two sublineages, A1a and B1a are the predominant haplotypes, which are geographically widespread, particularly near the zone of secondary

contact. Sublineages A1b and B1b were less frequent and found mainly near the ends of the transect.

When migration rates are high, populations are genetically well mixed, and the correlation between genetic distance and geographic distance is modest (Wilkins and Marlowe 2006). MtDNA migration rates indicated that gene flow within lineages A1 and B1 occurred at a higher rate than between lineages. Thus, it gave rise to lower mtDNA divergences within lineages A1 (2.3%) and B1 (1.4%) than between lineages A1 and B1.

Although low dispersal appears to play a role in determining the mtDNA distribution along the SE transect, it is unknown why there is such a low level of mtDNA admixture and why mtDNA gene flow between lineage A1 and B1 is asymmetric. It is possible that hybrids harbouring mtDNA lineage B1 in a putative nuclear background that corresponds to mtDNA lineage A1 could be less fit, and vice versa. Hence the next part of this study will address nuclear loci within this species in order to examine this and other hypotheses.

Chapter 3 Patterns of morphological variation across the mtDNA contact zone

3.1 Introduction

The role of morphology is prominent in studies of speciation. Speciation is usually associated with discrete morphological character differences between groups of organism (e.g. Brochu and McEachran 2000; Bauer et al. 2007). In contact zone studies, when diagnosable morphological and molecular characters appear to be concordant across broad geographic regions, it is often assumed that the two populations are independent evolutionary units (Matocq 2002). In addition, some morphological characters may be determinants of pre-mating isolation and thus can be important for mantaining contact zones between morphologically differentiated populations (Bensch et al. 1999; Rohwer et al. 2001).

Concordant patterns of morphological and genetic differentiation provide good evidence of reproductive isolation and speciation (Szymura and Barton 1991). Interspecific studies of zones of secondary contact generally report pronounced morphological differentiation, mostly accompanied by concordant genetic differentiation (e.g., Heaney and Timm 1985; Dessauer and Cole 1991; Szymura and Barton 1991; Glor et al. 2004; Fitzpatrick et al. 2008; Gligor et al. 2009). Intraspecific studies have also reported morphological differentiation in areas of secondary contact between previously isolated populations, where, in some cases, spatial patterns of morphological differentiation are concordant with mtDNA distributions (Fleischer et al. 1991; Arntzen and Wallis 1999; Good and Sullivan 2001; Bates et al. 2004).

However, several intraspecific studies also report discordant patterns of morphology and mtDNA (Cronin 1992; Brower 1996; Wiens and Penkrot 2002; Toon et al. 2003; Yang and Kenagy 2011). Biased gene introgression, extensive hybridization, and sexual selection are among several reasons used to explain this phenomena (García-París et al. 2003; Babik et al. 2005; Leaché and Cole 2007; Hird and Sullivan 2009; Renoult et

al. 2009). Moreover, some intraspecific studies report genetic differentiation between populations on either side of the secondary contact zone with no clear pattern of morphological differentiation (Brower 1994; Stone and Cook 2000). In these cases, it is sometimes suggested that convergent selection regimes on either side of the contact zone have overridden the effects of allopatric divergence and left no trace on morphology (Thorpe et al. 1996; Johansson et al. 2008a).

Selection is one of the factors proposed to maintain the contact zone (Jiggins and Mallet 2000; Dasmahapatra et al. 2002). In particular, sexual selection resulting from morphological differentiation between populations may play an important role (Rolán-Alvarez et al. 1999). For examples, assortative mating has been associated with different colour patterns in *Heliconius* butterflies (Mallet et al. 1998), body sizes and colour in birds (Cicero 2004; Gay et al. 2007) and newts (Babik et al. 2003), and sexual organs in wood rats (Matocq 2002) and fruit flies (Coyne 1993). Analyses of morphological differentiation across the contact zone may therefore help provide a better understanding of the persistance of distinct lineages.

In this chapter, I aimed to assess whether the geographical distributions of *T. boettgeri* mtDNA lineages A1 and B1 are accompanied by morphological changes. Avise (1987) suggested that mtDNA variation may not correlate with other aspects of the genome because of selection, introgression, poor resolution of the data, or lineage sorting. However, if mtDNA lineages are indicative of separate species rather than intraspecific variation, then this should have a significant effect on morphological character states and nuclear DNA (Bradley and Baker 2001). Since it is possible that mtDNA may cause genetic incompatibility, which may eventually lead to speciation, then individuals from different mtDNA lineages should show distinct morphological characters. If strong reproductive barriers exist between these mtDNA lineages, then concordant geographical

patterns of morphology and mtDNA are expected. Alternatively, hybrids may be observed at the contact zone and sites where mtDNA admixture occurs, suggesting significant nuclear gene flow between individuals with different mitochondrial types.

This study will also be useful to determine whether morphological differentiation plays an important role in maintaining the contact zone. Baack and Rieseberg (2007) suggested that when diverged populations come into contact, the extent of introgression between them is related to the degree of differentiation that they have accumulated. As mentioned in Chapter 2, there is relatively little mtDNA admixture at the zone of secondary contact. Therefore, if differentiated morphological characters between northern and southern populations are found, they may act as pre-mating barriers. If this is the case, hybrids should be rare and restricted only within the contact zone.

3.2 Methodology

3.2.1 Morphological characters

All individuals in this analysis are from the SE transect (locations of sample sites on this transect are described in Chapter 2). Only individuals large enough to sex were included in the analyses. Sex was established by hemipenal inversion and head shape examination. Large sample sizes were obtained (males $n = 169$, females $n = 226$) to allow detection of subtle morphological trends. Seven morphological characteristics, that have already been shown to vary geographically within *Tarentola* spp. (Thorpe 1991; Gübitz et al. 2000; Gübitz et al. 2005), were selected (Table 6). Measurements were taken on live animals. Each character was recorded three times to minimize measurement error. The body dimensions were measured in millimetres using a vernier caliper accurate to two decimal places. Scalation characteristics were counted under the light microscope before releasing the animals at the original locations.

3.2.2 Statistical methods used to determine pattern of morphological diversity of T. boettgeri

Analyses were carried out using SPSS (version 17, IBM Corporation). Morphological characters were tested for group (site) and sex differences. Kolmogorov-Smirnov of normality and Levene's test of homogeneity of variances were used to determine whether the data met the main assumptions of the ANOVA. Scalation characters and body size (snout-vent length) were analyzed using one-way analysis of variance (ANOVA) to assess whether there were significant differences among sites. Nutrition and ecological conditions (i.e., population density and food abundance) may affect the growth of animal and cause differences between populations. To avoid this problem, head length and head depth were adjusted for body size using analysis of covariance (ANCOVA).

Discriminant Function Analyses (DFAs) were used to obtain generalized patterns of variation across sites. To determine the effects of sexual dimorphism on morphological characters in this species, I treated the sexes separately. Variation was examined using plots of mean function scores against geographical position on the transition zone. It should be noted that these plots are intended to depict general phenetic grouping patterns based on the overall among-site morphological variations.

Principal component analysis (PCA) is an unconstrained ordination technique that is best used with correlated data to produce new uncorrelated variables that represent the main features of the original data. To clarify relationships between morphological data and mitochondrial DNA at the putative contact zone, PCA was used to discriminate morphological differences between individuals from 3 sites near the centre of the transect zone, containing 3 mitochondrial haplotype clades (A1, B1 and B2). Male and female morphological data were combined. In this analysis, the most variable factor scores

(normalized so that the pooled within-group standard deviation is unity) are plotted against each other to obtain a graphical representation of patterns of variation within the group.

Table 6 Quantitative Trait Data Collection.

Category	Trait	Abbrev.	Description
Body dimensions	Snout-vent length	SVL	Anterior tip of the snout to the anterior edge of the vent
	Head length	HL	Ear to the anterior tip of the snout
	Head depth	HD	At the posterior edge of the eye
Scalation	Large lamellae	LAL	Number of large lamellae (at least two times as wide as long) on the fourth toe of right hind foot
	Pre-cloacal scales	PRECL	Counted from leg axis to cloaca
	Upper labial scales on right and left sides	UPLAB	Counted from the middle of the eye to the mental scale (excluding the mental)
	Lower labial scales on right and left sides	LOLAB	

Note: See Figure 15 for more detail

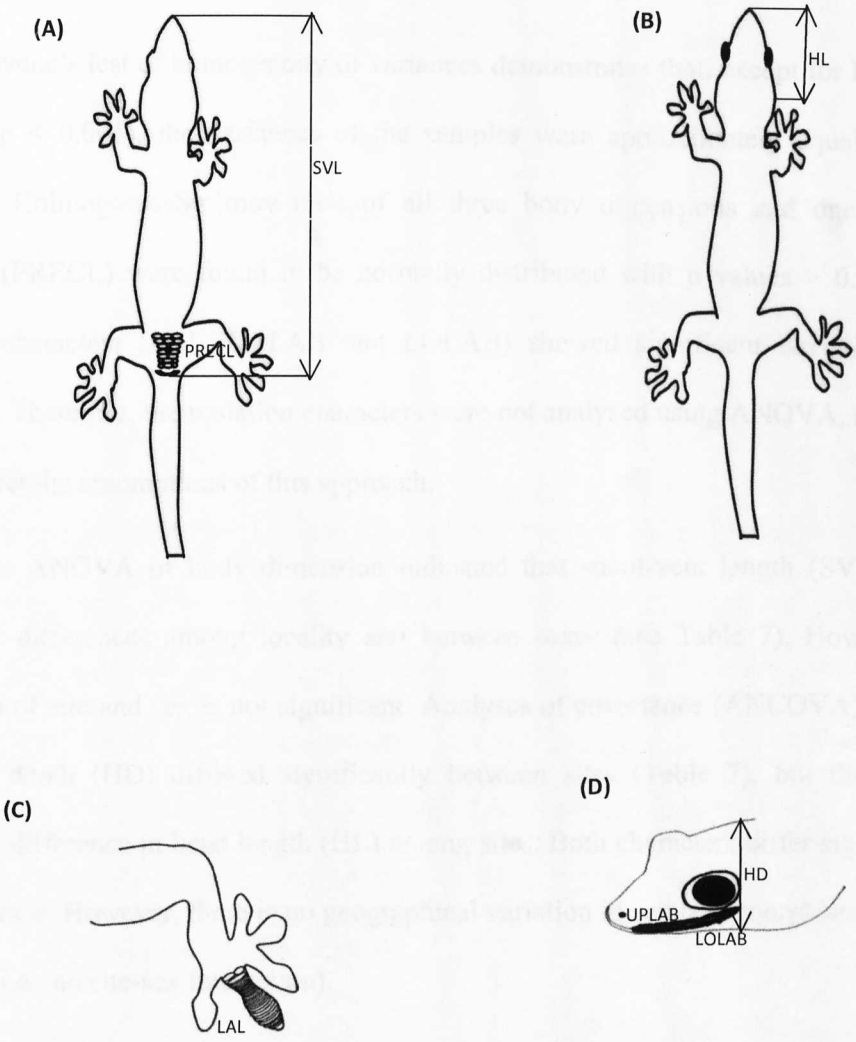


Figure 15. Schematic diagrams showing morphological characters recorded in *T. boettgeri*. (A), (B) are ventral and dorsal view, respectively. (C) Ventral view of the right hide foot. (D) Head view of *T. boettgeri*.

3.3 Results

3.3.1 Variation in morphological characters between groups and sexes

Levene's test of homogeneity of variances demonstrates that, except for PRECL ($F = 4.523$, $p < 0.001$), the variances of the samples were approximately equal for each character. Kolmogorov-Smirnov tests of all three body dimensions and one scalation character (PRECL) were found to be normally distributed with p values > 0.05. Three scalation characters (LAL, UPLAB and LOLAB) showed significant deviations from normality. Therefore, the scalation characters were not analysed using ANOVA, since they did not meet the assumptions of this approach.

The ANOVA of body dimension indicated that snout-vent length (SVL) shows significant differences among locality and between sexes (see Table 7). However, the interaction of site and sex is not significant. Analyses of covariance (ANCOVA) revealed that head depth (HD) differed significantly between sites (Table 7), but there is no significant difference in head length (HL) among sites. Both characters differ significantly between sexes. However, there is no geographical variation in sexual dimorphism in either character (i.e., no site-sex interaction).

3.3.2 Discrimination function analysis of T. boettgeri

The DFAs revealed that the patterns of among-site variation were quite multidimensional. As a result, the first three DFs for both males (55.0%, 18.2% and 13.9% of variance) and females (54.9, 16.7% and 12% of variance) were used for further analysis of variation between-groups and classification of individuals (Table 8). One-sample Kolmogorov-Smirnov tests for normality revealed that all DFs were normally distributed.

Table 7 Summary of ANOVA and ANCOVAs of body dimension characters. The F-ratio for the ANOVA on SVL and a two-way ANOCVA for HL and HD are given. The factors are site (df = 13, 366) and sex (df = 1, 366). P values less than 0.05, 0.01 and 0.001 are represented by the letters a, b and c, respectively.

Variable	Locality	Sex	Locality*Sex
SVL	3.10^{c}	24.04^{c}	1.22^{ns}
HL	1.06^{ns}	11.73^{b}	1.18^{ns}
HD	3.89^{c}	14.24^{c}	1.65^{ns}

Table 8 Summary of variable loading for DFA. Structure matrix (pooled within-group correlations between discriminating variables and standardized canonical discriminant functions) 1, 2 and 3 are shown for males (M) and females (F). Variables are ordered by absolute size of correlation within function. The asterisk (*) represents largest absolute correlation between each variable and any DF. Canonical discriminant functions, eigenvalues, percent variance, cumulative percent variance and squared canonical correlation (canonical correlation) are given.

	DF1		DF2		DF3	
Structure Matrix	M	F	M	F	M	F
SVL	-0.680*	0.883*	0.414	0.121	0.374	0.101
HL	0.339	0.196	0.805*	0.615*	0.323	-0.402
HD	0.204	0.210	0.491	0.079	0.741*	0.606*
LAL	0.129	-0.034	0.594	0.581	0.628*	-0.250
PRECL	-0.080	0.267	-0.032	-0.246	0.121	0.053
UPLAB	0.199	-0.068	0.166	0.044	0.322	0.068
LOLAB	0.139	0.322	-0.075	-0.193	0.135	0.050
Eigenvalue	0.833	0.589	0.276	0.179	0.211	0.129
% of variance	55.0	54.9	18.2	16.7	13.9	12.0
Cumulative %	55.0	54.9	73.3	71.6	87.2	83.6
Canonical correlation	0.674	0.609	0.465	0.390	0.417	0.338

DF scores were examined by mtDNA lineages. Since males and females are different, each sex was analysed separately. Mean DF scores of individuals belonging to mtDNA lineage A1 and B1 were calculated separately and plotted against distances from the centre of the contact zone. Mean DF were plotted to show morphological variation along the transect (Figure 16 and Figure 17). Individuals belonging to mtDNA lineages A2 and B2 were plotted separately in order to determine whether they were differentiated from the majority of individuals (which belong to mtDNA lineage A1 and B1). In general, DF plots revealed no distinguishable pattern of variation between the north and south of the contact zone for either males or females.

For male DF1, northern populations (mtDNA lineage B1) exhibited slightly lower means than those of southern populations (mtDNA lineage A1). This is except for three sites (site 01, 07 and 14) at the centre of the transect, which show high means (see Figure 16A). At site 07, the 95% confidence interval of individuals from mtDNA lineage A1 slightly overlapped with that of lineage B1, but was still distinguishable. At site 01, DF1 scores of two individuals belonging to lineage B2 are not similar. Within southern populations, DF1 scores were highest at site 02, but appear to be lowest for site 12 and 13 (Figure 16A).

For male DF2 and DF3 each individual was plotted separately at site 07 due to highly variable DF scores among population from lineage A1. In both cases, patterns of variation are not distinguishable between northern and southern populations. Nevertheless, means of site 02 are also clearly differentiated from other sites. Moreover, mean DF3 at site 07 is highest of all northern populations.

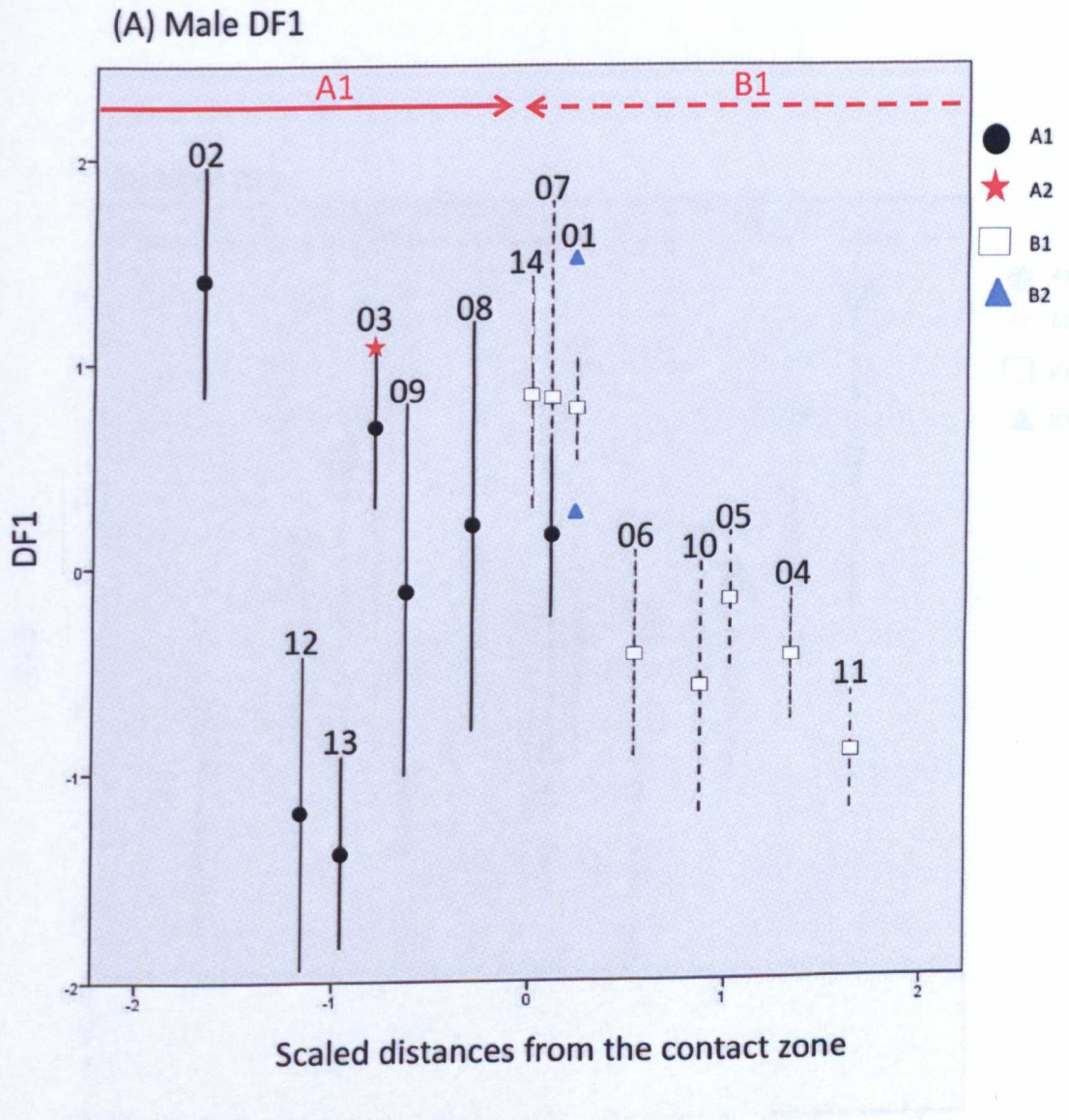


Figure 16A. Means and 95% confidence intervals for DF1 for males. Means of individuals classified by mtDNA lineage across the sites. Means were plotted based on location of sampling sites (numbers above bars), from south (-) to east (+) of the contact zone.

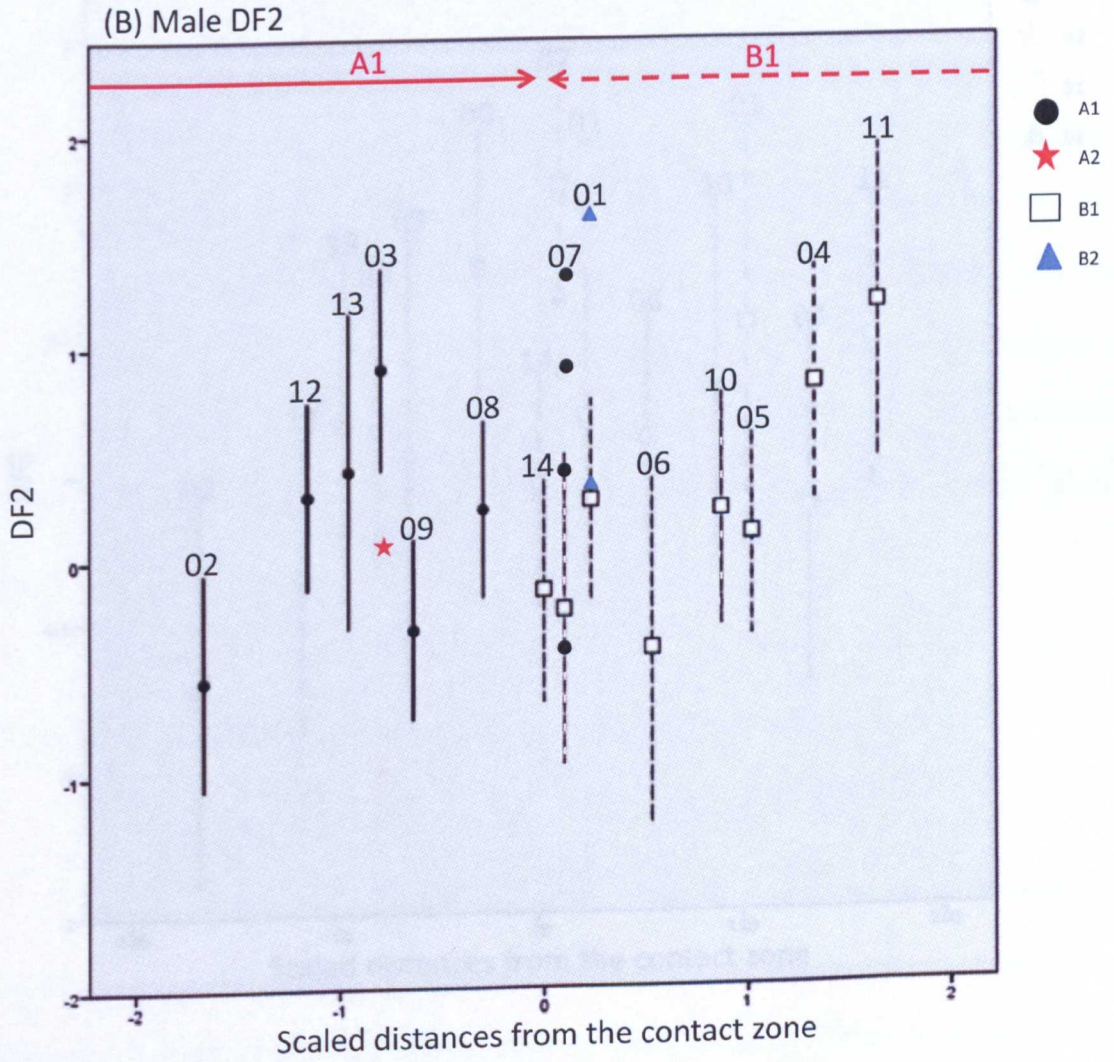


Figure 16B. DF2 for male, see Figure 16A for details.

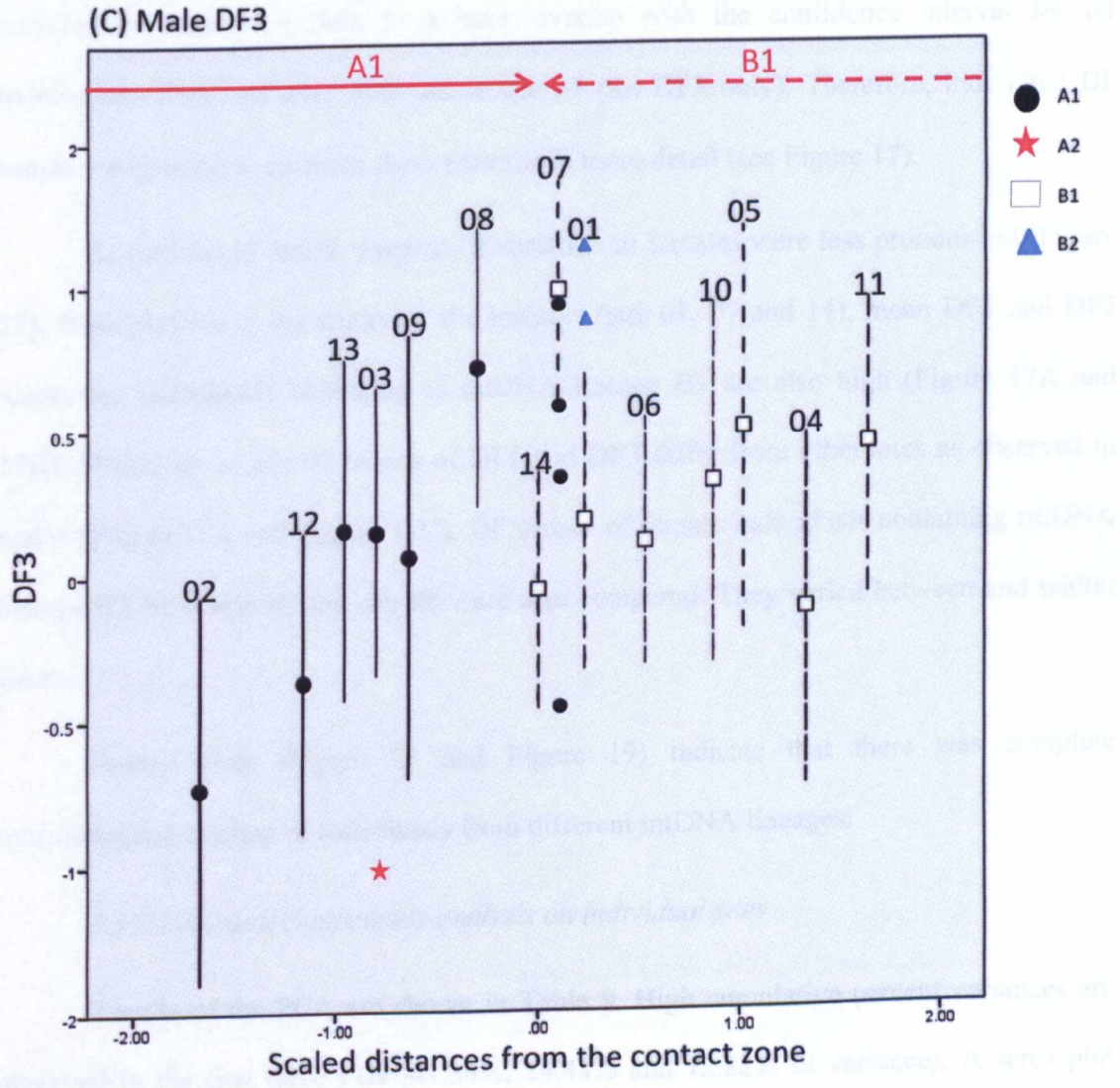


Figure 16C. DF3 for male, see Figure 16A for information.

For females, at site 01, individuals classified into mtDNA lineage A1 have highly variable DF scores, leading to a large overlap with the confidence interval for B1 individuals. This was also observed at site 07 (for DF3 only). Therefore, individual DF scores was plotted to examine these patterns in more detail (see Figure 17).

In contrast to males, patterns of variation in females were less pronounced (Figure 17). Nevertheless, at the centre of the transect (site 01, 07 and 14), mean DF1 and DF2 scores for individuals belonging to mtDNA lineage B1 are also high (Figure 17A and 17B). Moreover, at site 02 means of DF1 and DF3 differ from other sites as observed in males (Figure 17A and Figure 17C). DF scores of female individuals containing mtDNA lineage B2 from site 01 and site 06 were also compared. They varied between and within sites.

Scatter plots (Figure 18 and Figure 19) indicate that there was complete morphological overlap of individuals from different mtDNA lineages.

3.3.3 Principal components analysis on individual sites

Results of the PCA are shown in Table 9. High cumulative percent variances are observed in the first three PCs (41.30%, 24.44% and 12.82% of variance). A scree plot between eigenvalue and component number showed a dramatic decline in variation from component 3 onward, which corresponds to expression of cumulative variance (data not shown). The scatter plot of PC scores 2 against 1 and 3, respectively, were shown in Figure 20. It did not appear possible to morphologically discriminate between individuals with different mitochondrial haplotypes.

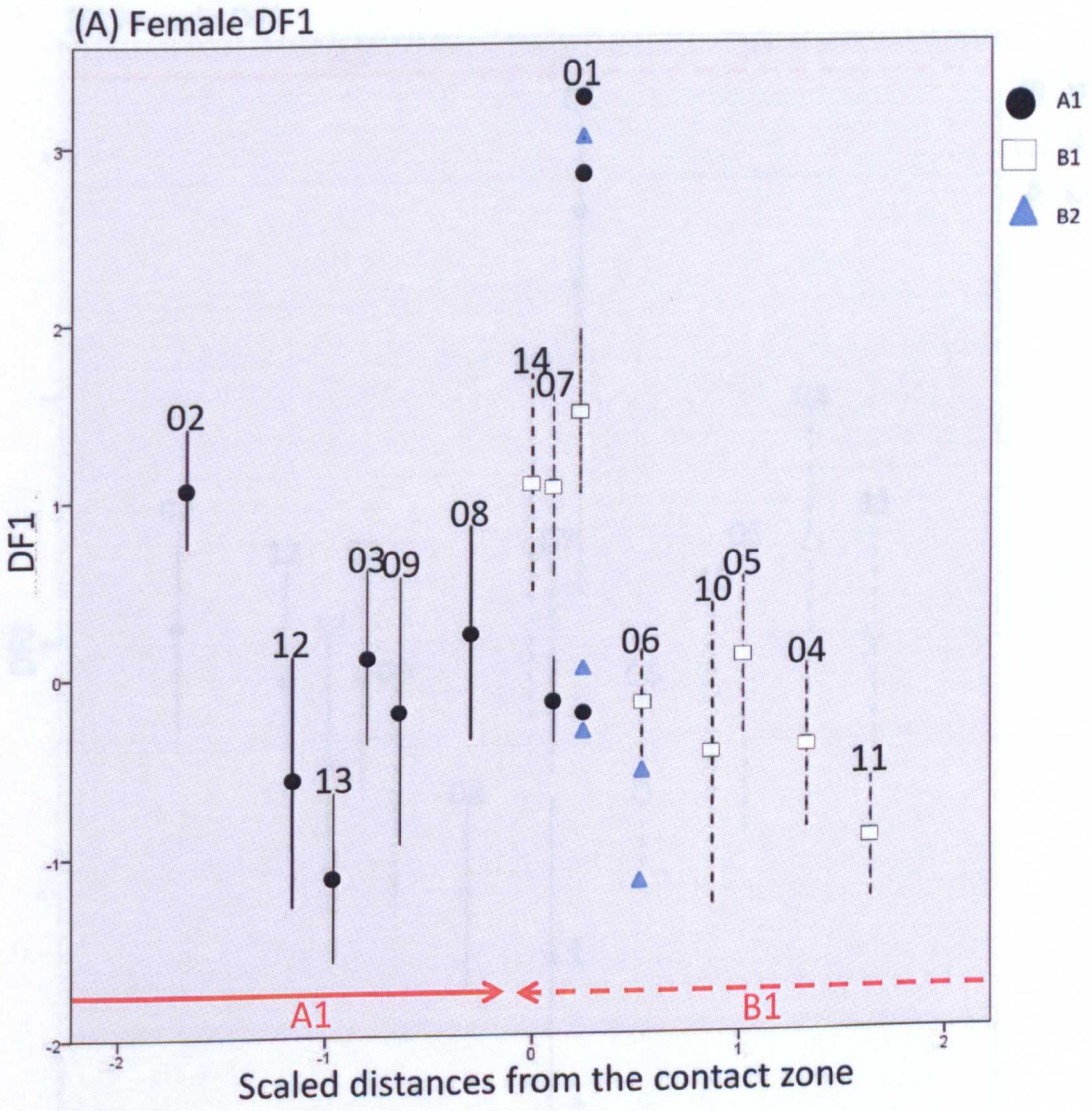


Figure 17A. DF1 for females, see Figure 16A for information.

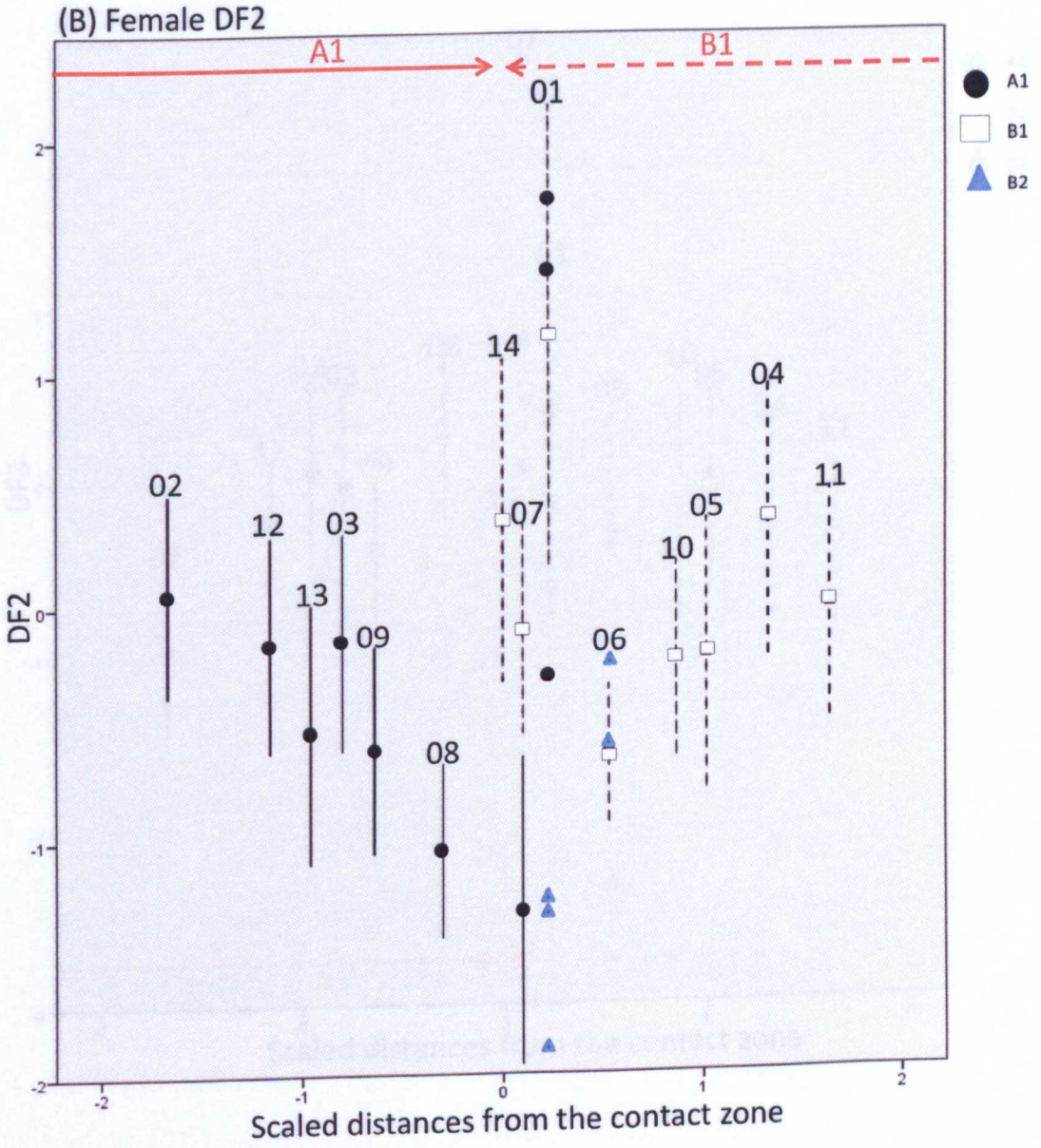


Figure 17B. DF2 for females, see Figure 16A for information.

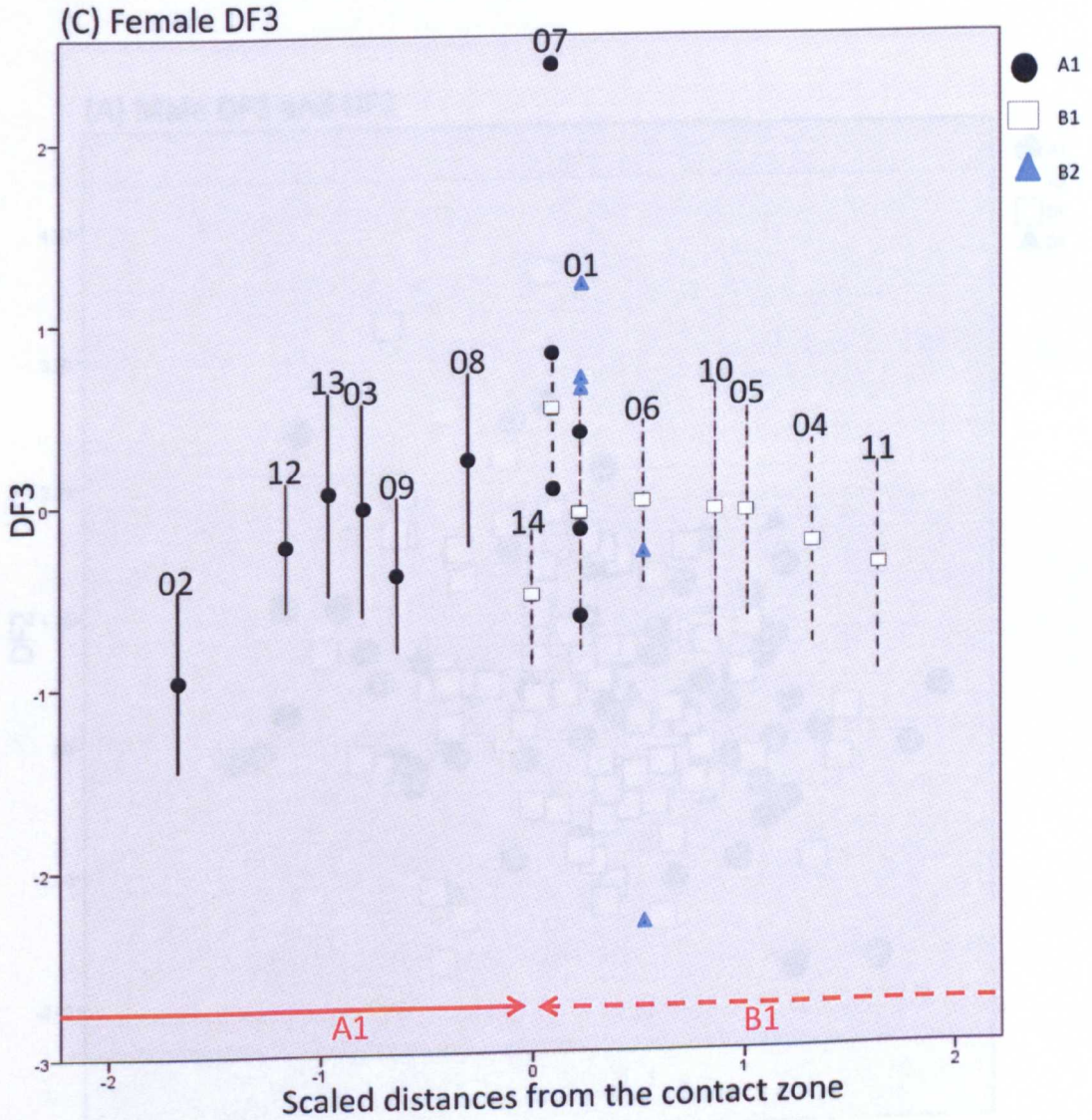


Figure 17C. DF3 for females, see Figure 16A for more information.

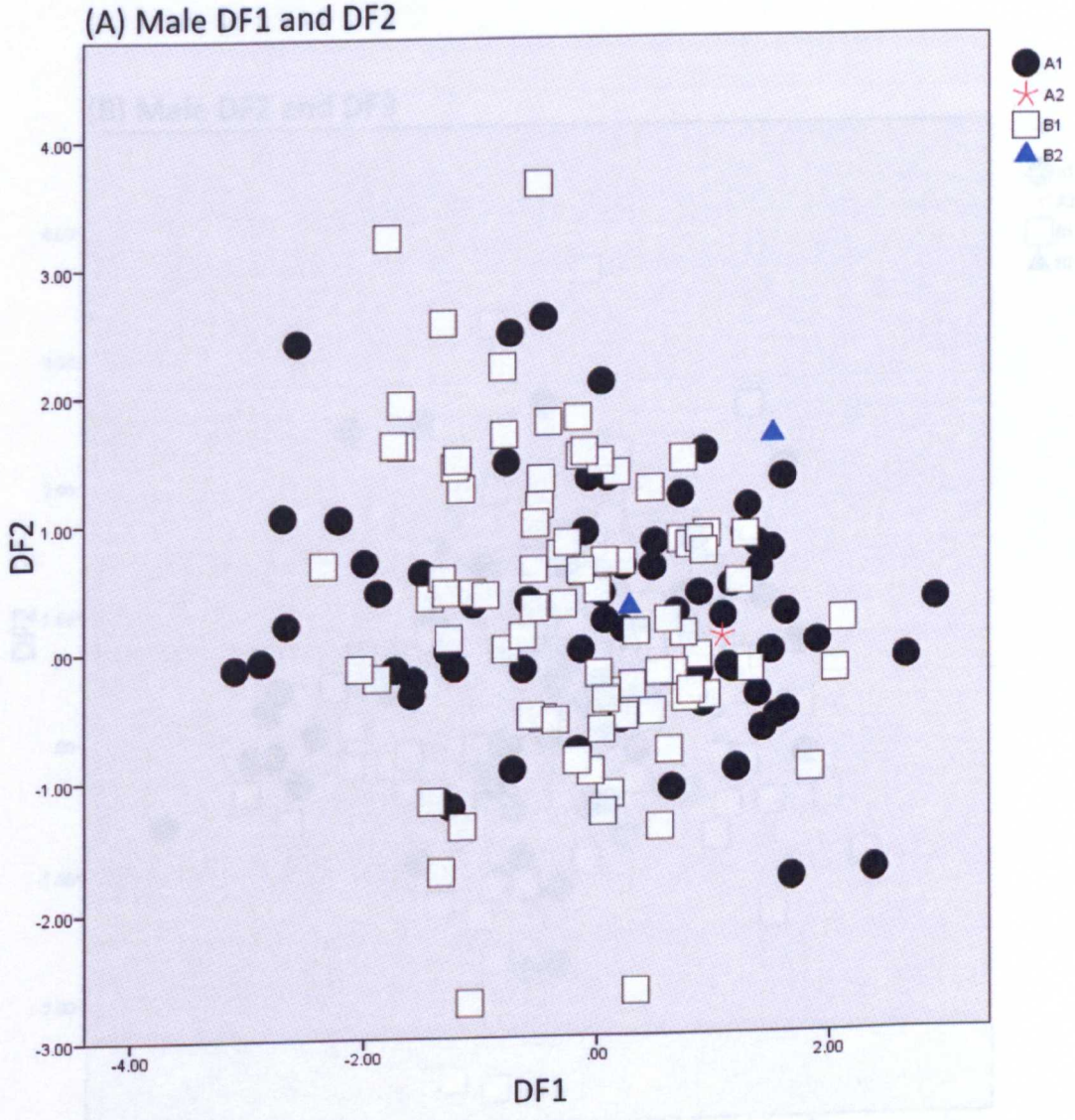


Figure 18. Scatter plots of male DF scores: (A) DF1 against DF2 and (B) DF2 against DF3. Individuals are labelled according to mtDNA lineages: circle (A1), star (A2) box (B1) and triangle (B2).

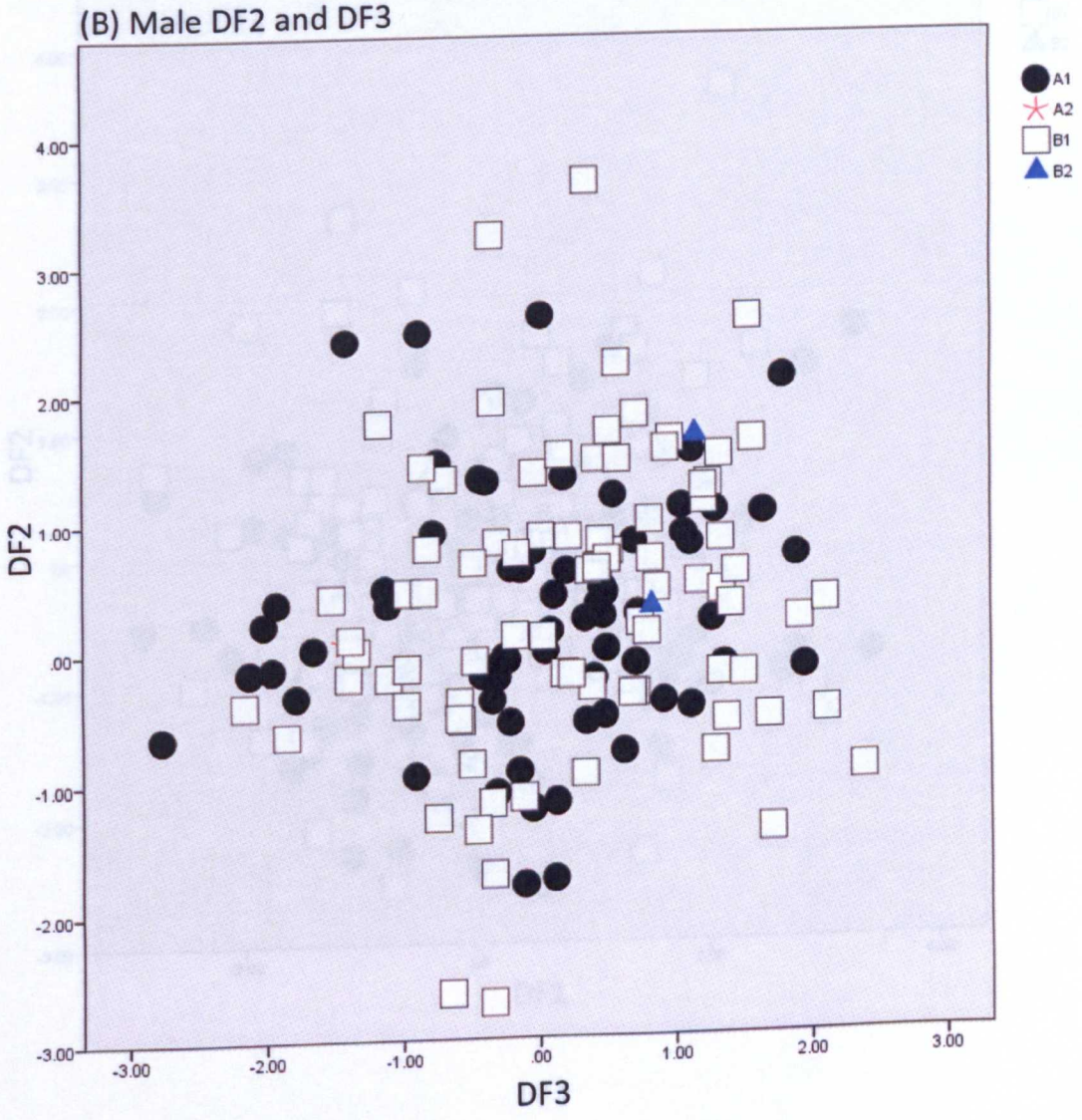


Figure 18 (cont.)

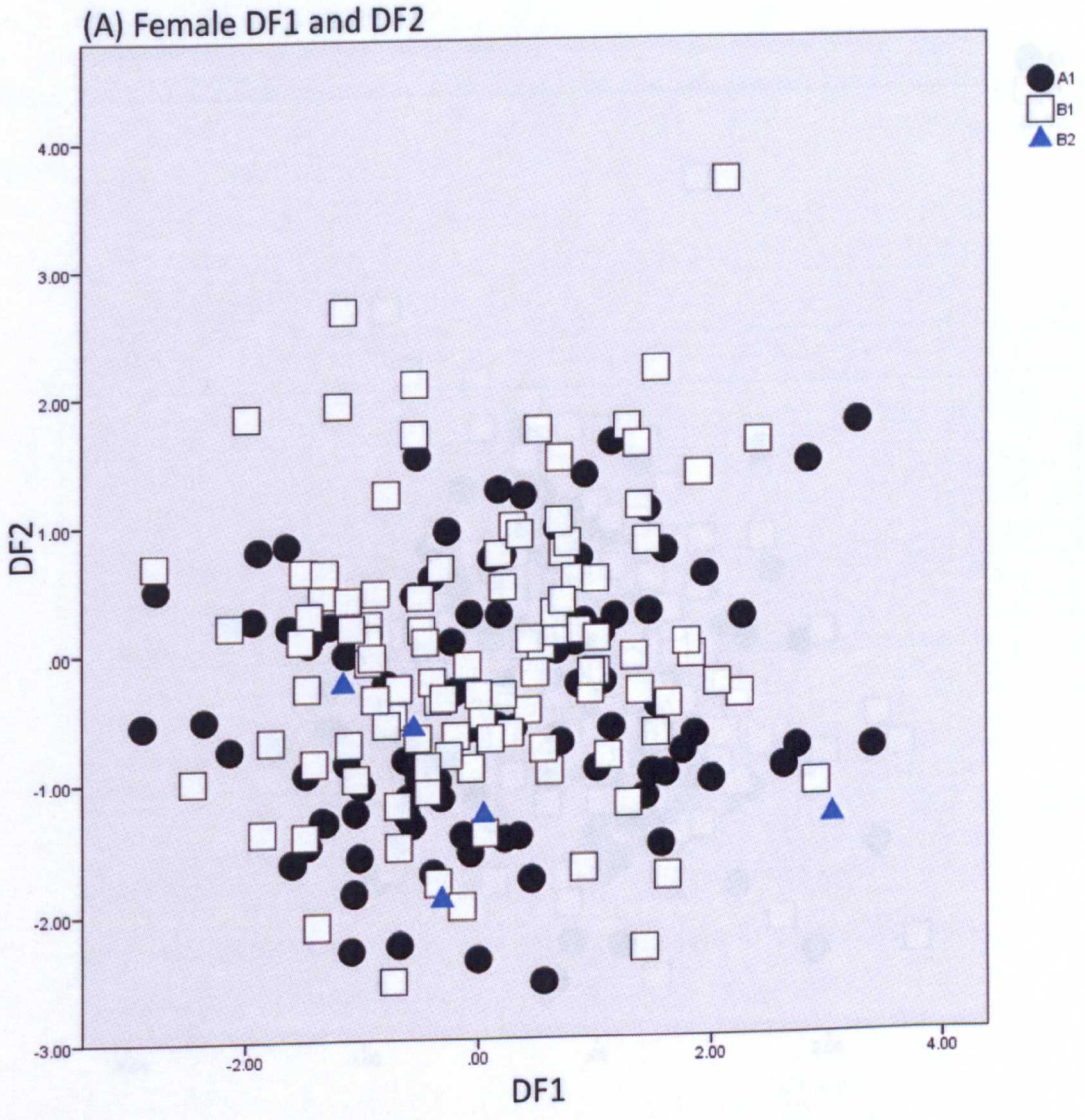


Figure 19. Scatter plots of female DF scores (A) DF1 against DF2 and (B) DF2 against DF3. Individuals classified into mtDNA lineages A1, B1 and B2 are represented as circle, box and triangle, respectively.

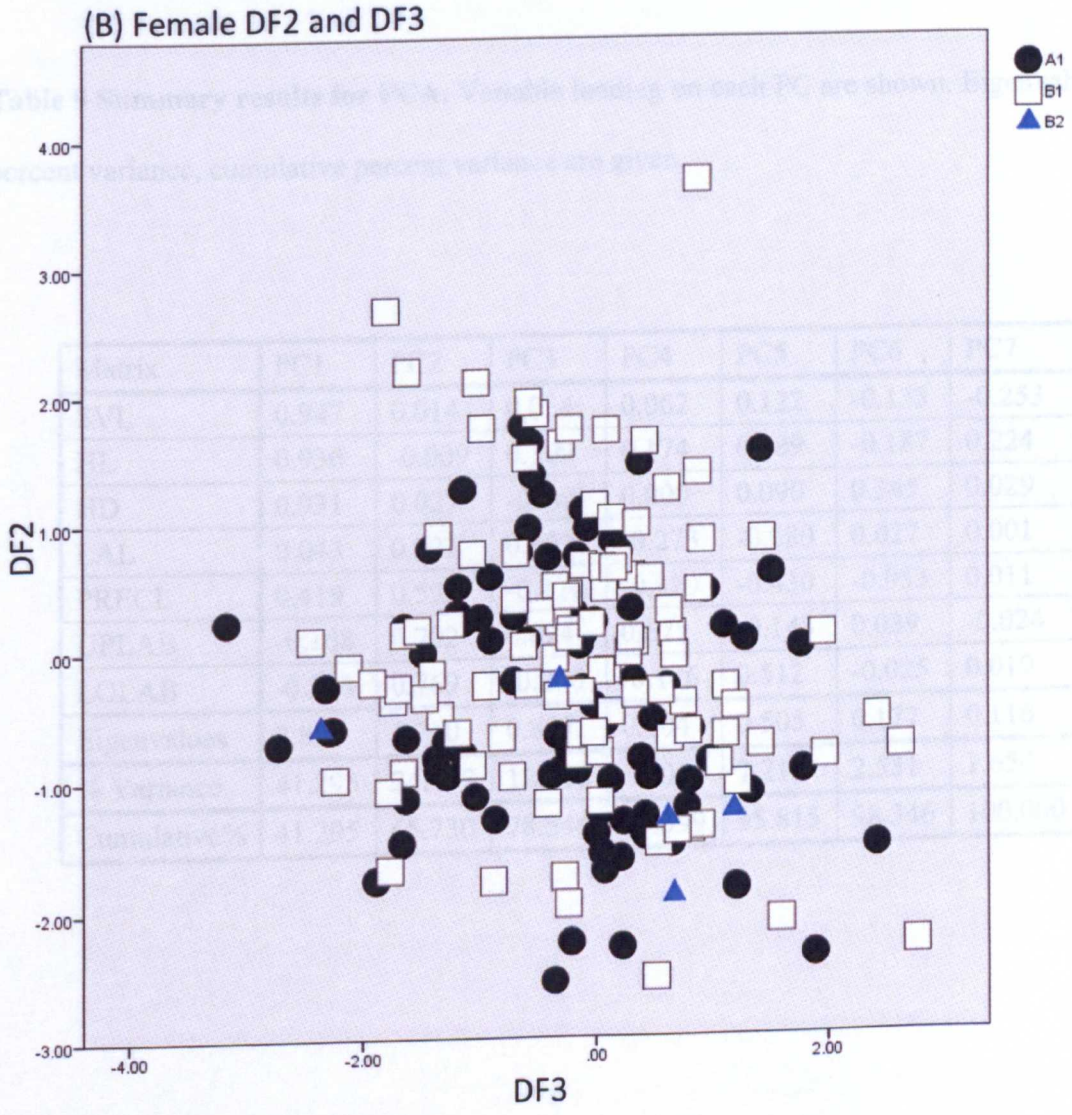


Figure 19 (cont.)

Table 9 Summary results for PCA. Variable loading on each PC are shown. Eigenvalues, percent variance, cumulative percent variance are given.

Matrix	PC1	PC2	PC3	PC4	PC5	PC6	PC7
SVL	0.947	0.014	0.054	0.062	0.122	-0.133	-0.253
HL	0.930	-0.009	0.109	0.174	0.089	-0.187	0.224
HD	0.931	0.027	-0.069	0.000	0.090	0.345	0.029
LAL	0.043	0.523	0.802	-0.273	-0.080	0.027	0.001
PRECL	0.419	0.593	-0.420	-0.329	-0.430	-0.053	0.011
UPLAB	-0.168	0.702	0.024	0.675	-0.143	0.039	-0.024
LOLAB	-0.238	0.769	-0.240	-0.176	0.512	-0.025	0.019
Eigenvalues	2.891	1.710	0.897	0.704	0.505	0.177	0.116
% Variance	41.295	24.435	12.816	10.054	7.215	2.531	1.654
Cumulative%	41.295	65.730	78.546	88.599	95.815	98.346	100.000

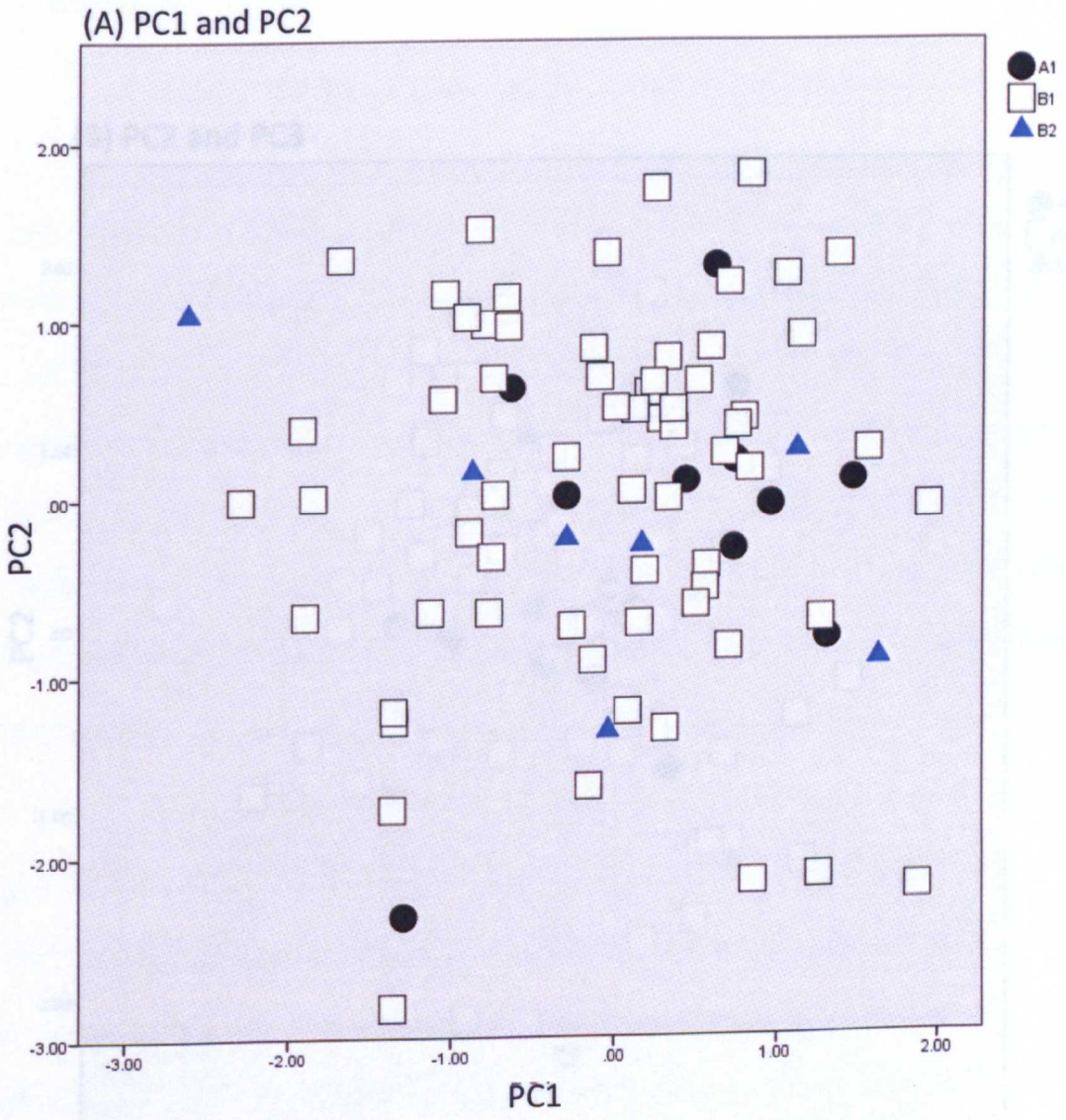


Figure 20. Scatter plots of principal component scores (PCs). (A) is PC1 against PC2. (B) is PC2 against PC3. Symbols denote mtDNA lineages.

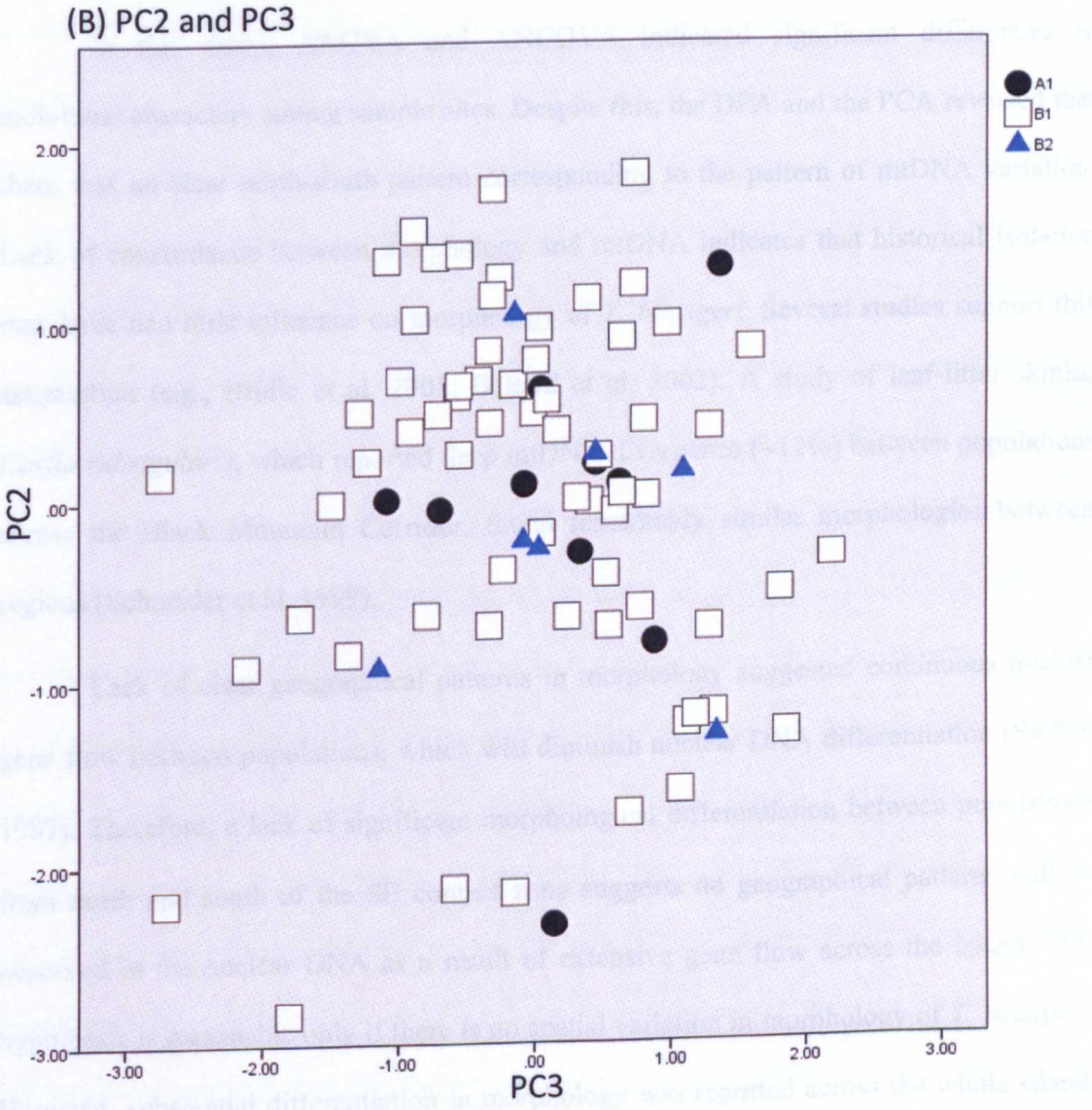


Figure 20 (cont.)

3.4 Discussion

3.4.1 Pattern of morphological variation across the mtDNA transition zone

In this study, ANOVA and ANCOVA indicated significant differences in individual characters among sample sites. Despite this, the DFA and the PCA revealed that there was no clear north-south pattern corresponding to the pattern of mtDNA variation. Lack of concordance between morphology and mtDNA indicates that historical isolation may have had little influence on morphology of *T. boettgeri*. Several studies support this assumption (e.g., Bridle et al. 2001; Ballard et al. 2002). A study of leaf-litter skinks, *Carlia rubrigularis*, which reported deep mtDNA divergence (~12%) between populations across the Black Mountain Corridor, found remarkably similar morphologies between regions (Schneider et al. 1999).

Lack of clear geographical patterns in morphology suggested continuous nuclear gene flow between populations, which will diminish nuclear DNA differentiation (Slatkin 1987). Therefore, a lack of significant morphological differentiation between populations from north and south of the SE contact zone suggests no geographical patterns will be observed in the nuclear DNA as a result of extensive gene flow across the island. This hypothesis is reasonable only if there is no spatial variation in morphology of *T. boettgeri*. However, substantial differentiation in morphology was reported across the whole island, although the geographical patterns do not correspond to the distribution of mtDNA lineages (see Gübitz et al. 2005). Therefore, the results obtained in this study merely indicate that mtDNA and nuclear DNA spatial patterns are discordant, and this is reflected in this morphological study.

Divergence in morphology may have occurred in response to spatially variable natural selection as was reported for several volcanic island endemics (Fritts 1984; Báez and Brown 1997; Malhotra and Thorpe 2000; Filardi and Smith 2005; Wolf et al. 2008),

including Gran Canaria (Brown and Thorpe 1991; Thorpe and Báez 1993). Previous studies of *T. boettgeri* also suggested that morphological variation has arisen in response to ecological diffrerences (i.e., arid, humid, altitude and vegetation) within the island (Nogales et al. 1998; Gübitz et al. 2005). If this hypothesis is true, morphology at the SE transect should be uniform due to a lack of environmental differentiation (Garcia Rodriguez et al. 1990; Brown 1991). Nevertheless, some sampling sites (particularly at site 01, 02 and 07) showed pronounced morphological difference, regardless of their mtDNA backgrounds. This suggested that morphology of individuals from those sampling sites might be influenced by other factors, such as difference in nuclear DNA backgrounds.

3.4.2 Role of morphology in maintaining the contact zone

Lack of distinct phenotypic traits suggests that morphology does not contribute to the maintenance of the mtDNA contact zone. It also indicates that it is probably unlikely that the mtDNA transition is due to any restriction of migration or gene flow between populations on the transect, otherwise morphology would show a similar pattern. It also suggests no corresponding nuclear DNA contact zone exists, and therefore the mtDNA contact zone is unlikely to be maintained by cyto-nuclear incompatibility. Nevertheless, information from interspecific studies, where concordant spatial patterns of nuclear and mtDNA were reported, demonstrate that cyto-nuclear incompatibilities are not necessarily coupled with pronounced morphological differences (e.g., Sweigart et al. 2006; Fritz et al. 2008; Pryke and Griffith 2008; Furman et al. 2010). This could be confirmed by demonstrating an association between nuclear and mtDNA genotypes across individuals. This will be examined through detailed analyses of multilocus nuclear DNA in the next chapters.

Chapter 4 Identification and development of microsatellite markers

4.1 Introduction

This chapter is based on a manuscript that has been published (Appendix 3): 'Tejangkura, T and Brown, RP. Ten novel microsatellite loci from the Gran Canarian gecko, *Tarentola boettgeri*, and their applicability in other *Tarentola*. Molecular Ecology Resources Database 1 April 2010 – 31 May 2010 Volume 10, Issue 6, pages 1098–1105, November 2010'

Phylogeographical analysis of *T. boettgeri* within Gran Canaria detected five major mtDNA lineages, which appear to have been on separate evolutionary trajectories for several million years, consistent with patterns of volcanism within the island. This finding makes *T. boettgeri* an interesting model for examining putative speciation on a microgeographic scales. However morphological variation is not consistent with the pattern of mtDNA divergence (Gübitz et al., 2005; this thesis). Since morphological variation may or may not reflect underlying variation in the nuclear DNA, it was also of interest to examine nuclear DNA markers to determine whether the phylogeographical break observed in the SE of the island corresponded to a similar transition in the nuclear genome. Recent methodological advances have facilitated the use of multilocus data to reconstruct the evolutionary history of diverging populations, which can provide a means for testing demographic models of speciation and quantifying biological relevant parameters underlying species limits (Good et al. 2008). Therefore, microsatellites have emerged as the most popular and versatile marker type for ecological applications (Selkoe and Toonen 2006).

Microsatellites (or Short Tandem Repeats: STRs) are repetitive sequences of mostly 2 to 4 nucleotides with a widespread occurrence particularly in multicellular organisms (Ramel 1997). They appear to be more or less uniformly distributed across eukaryotic genomes, but are under-represented in coding regions, and perhaps telomeres

(Goldstein and Schlötterer 2001). Microsatellites are thought to arise by the process referred to as DNA slippage or slipped strand mispairing (Bennett 2000). This slippage is thought to occur within the DNA-DNA polymerase complex, which mediates DNA replication, as a consequence of mispairing (by one or occasionally more repeat unit) between the original template strand and the newly synthesised DNA strand. The region of unpaired DNA is then formed a loop (Figure 21). If the loop is on the new strand, the net effect is addition of a repeat unit. If this is on the template strand, it is removed by enzymatic proofreading and the net effect is the loss of a repeat unit. It should be noted that microsatellite mutations are the interaction between slippage and mismatch repair mechanism maintaining the fidelity of DNA replication. Thus, if the observed DNA mutation rate is low, microsatellites would be very rare and less polymorphic. If they are high, many would show changes from one generation to the next, preventing pattern of inheritance being investigated accurately (Bennett 2000).

The uses of microsatellite loci has become widespread in studies of relatedness, paternity and population structure due to their abundance in eukaryotic genomes, high polymorphism, and amenability to PCR technology (Goldstein and Schlötterer 2001). Microsatellite sequences are considered the most revealing DNA markers available for inferring population structure and dynamics (Zhang and Hewitt 2003). Many highly polymorphic microsatellites of various types of repeat have been reported for reptiles (e.g. Gardner et al. 1999; Bloor and Dávila 2008), some with potentially cross-species uses (e.g., Bloor et al. 2006; Suárez et al. 2008). However, no microsatellites have been reported for the genus *Tarentola*. Therefore, another aim of this thesis is the development of polymorphic microsatellite markers specific to *T. boettgeri*, will be useful to further population investigation by allowing analyses of nuclear DNA.

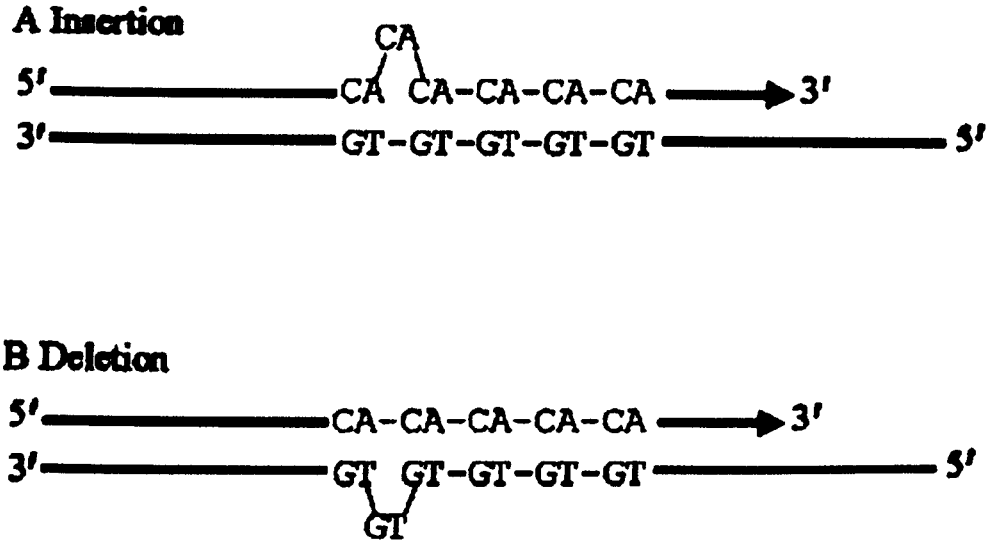


Figure 21. Strand slippage during DNA replication. (A) The extending strand slips backwards, resulting in the insertion of an extra repeat within the newly synthesised strand. (B) The extending strand slips forward, resulting in the deletion of a repeat within the newly synthesised strand. (From Bennett 2000)

Our purpose here was to identify microsatellite markers for subsequent analysis of within-island contact zone of two *T. boettgeri* mitochondrial lineages A1 and B1. In this study, we used tetranucleotide microsatellite loci due to the greater stability in the genome and low strand slippage-errors than the commonly used dinucleotide and trinucleotide repeats (Ramel 1997). The tetranucleotide (AAAG)n microsatellite-enriched library was constructed and tested for the suitable nuclear DNA markers to identify genetic variation and transitional zone of *T. boettgeri* on Gran Canaria.

4.2 Methodology

4.2.1 Source of material and primers

Sources of genomic DNA, synthetic oligonuclotide primers and chemical reagents are as described in Chapter 2. The oligonucleotide primers used for construction of microsatellite-enriched library are listed in Table 10. The physical map of the high copy plasmid vector, pGEM-T Easy (Promega) used as a vector for construction of microsatellite-enriched library is shown in Figure 22. Genomic DNA of *T. boettgeri* was isolated as mentioned in Chapter 2.

4.2.2 Microsatellite Enriched Library Construction

Microsatellite loci were isolated from a partial genomic library enriched for the tetranucleotide $(AAAG)_n$ repeat sequence using an enrichment protocol modified from Gardner et al. (1999) and Bloor et al. (2006). Genomic DNA was isolated from a single *T. boettgeri* sampled near Pinor de Tamadaba (Tb), in the centre-west of Gran Canaria (corresponding to clade A3 in Gübitz et al. 2005). Five micrograms of genomic DNA was digested in a total volume of 20 µl with 5 units of the restriction endonuclease *Sau*3AI (New England Biolabs) at 37°C for 3 hrs and 5 µl of Sau3AI digested DNA was observed on 1.5% agarose gel. Only complete digested DNA was used for microsatellite library construction.

4.2.2.1 Generation of Sau3AI adaptor and ligation with digested DNA fragments

In order to amplify the unknown DNA fragments, Sau3AI adapter was added to both ends of the fragments. To generate an adaptor, 1.5 nmol of linker oligo S62 was pre-heated at 80°C before being hybridized to an equal amount (1.5 nmol) of linker oligo S61. The solution was removed to RT and allowed to cool slowly over 1 hr. Then, 5 μg of Sau3AI digested DNA was ligated to 0.9 nmol of the adapter in 1X DNA ligase buffer containing 40 units T4 DNA ligase (Promega) in a total volume of 200 μl at RT. This reaction mix was placed into a container and left to cool slowly to 4°C in the incubator. The DNA was ethanol precipitated, resuspended in 20 μl 0.1 X TE buffer pH 8.0 and electrophoresed on a 2% agarose gel. DNA fragments of a selected size range (300-1000 bp) were purified using a Gel Extraction Kit (Sigma Aldrich).

4.2.2.2 Magnetic Isolation of $(AAAG)_n$ Microsatellites

Subtractive hybridization was carried out with 3' biotinylated oligo $(AAAG)_6$ and bound to Streptavidin MagneSphere ParaMagnetic particles (Streptavidin MagneSphere ParaMagnetic kit, Promega). 100 μl of Streptavidin MagneSphere ParaMagnetic particles were resuspended and washed as per manufacturers recommendation and resuspended in 100 μl 5X SSC (1X SSC = 0.15M NaCl, 15 mM trisodium citrate) containing 200 pmol of Biotinylated oligo S64. The bead mixture was incubated for 15 min at RT, washed 3 times in 5X SSC and resuspended in 50 μl of 1X hybridization solution (0.5M NaCl, 4% w/v polyethylene glycol 8000) at 55°C.

Table 10 Oligonucleotide used in microsatellite enriched library construction and their descriptions.

Primer	T_m (°C)	Sequences of primer from 5' to 3'	Gene/ DNA element	References
Standard primers for DNA sequencing				
T7	38.8	TAATACGACTCACTATAGGG	T7 promoter, pGEM-T easy vector	Promega technical manual No. 042
SP6	30.6	TATTTAGGTGACACTATAG	SP6 promoter, pGEM-T easy vector	
M13F	41.7	GTTTTCCCAGTCACGAC	M13 primers, pGEM-T easy vector	
M13R	36.2	CAGGAAACAGCTATGAC		
Tetranucleotide $(AAAG)_n$ microsatellite-enriched library				
S61	61.7	GGCCAGAGACCCCAAGCTTCG	*Sau*3AI linker oligo A, Primer specific to *Sau*3AI	*Egernia stokesii* (Refseth et al. 1997; Gardner et al. 1999)
S62	67.3	pGATCCGAAGCTTGGGGTCTCTGG CC	Phosphorylated *Sau*3AI linker oligo B	
S64	N/A	$(AAAG)_6$[Biotin]	Biotinylated probe	
S6	47.4	$(AAAG)_6$	Primer specific to $(AAAG)_n$ element	

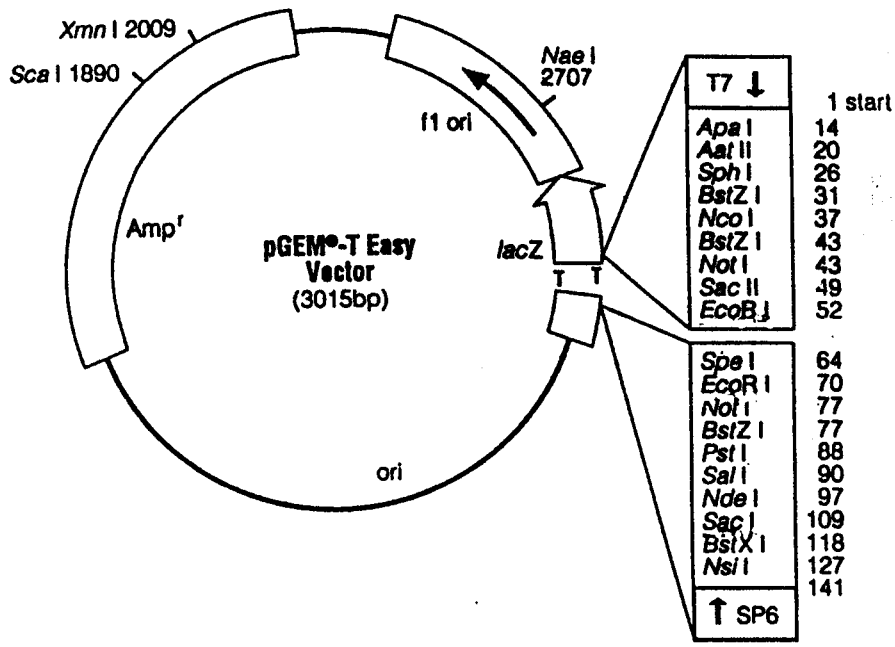


Figure 22. pGEM-T Easy Vector circle map and sequence reference points. (Picture taken from Promega, pGEM-T and pGEM-T Easy Vector Systems Promega technical manual No. 042)

In a separate tube, 10 µl of the ligated DNA/adapter solution was added to 40 µl of 1X hybridization solution which includes 20 pmol of S61. The mixture was heat-denatured at 95°C for 5 min and cooled to 55°C before adding all the resuspended bead mixture and incubated for 20 min at 55°C. The beads were then washed four times (in 100 µl of 2X SSC, 10 pmol S61) at RT and then washed 4 times in 100 µl 1X SSC, 10 pmol S61 at 30°C to remove unbounded DNA fragments. The captured DNA fragments were eluted from the beads by denaturing for 20 min at room temperature in 20 µl 0.15M NaOH. The solution was neutralized with 1.3 µl 1.25M acetic acid, and 2.2 µl 10X TE (pH 8.0) and DNA was purified using a Qiagen column (QIA quick PCR purification kit, QIAGEN).

4.2.2.3 PCR Amplification of Captured Fragments and Cloning into pGEM-T Vector

The enriched DNA (~280 ng) was amplified in a 50 µl volume containing 1X premix PCR buffer (Sigma Aldrich) and 30 pmol of S61. The amplification was carried out in a thermal cycler with one cycle of denaturing at 94°C for 3 min, annealing for 45 s at 60°C, extension for 1 min at 72°C followed by 39 cycles of 94°C for 45 s, 60°C for 45 s, 72°C for 45 s, ending in one cycle of 72°C for 5 min. The PCR product was purified using QIA quick PCR purification kit (QIAGEN) and eluted in 20 µl elution buffer. The purified PCR product was ligated into pGEM-T easy vector (Promega) following the modified protocol from Sambrook et al. (2000).

Plasmid-DNA ligation was carried out using T_4 DNA ligase (Invitrogen Inc.) that catalyzed the formation of phosphodiester bond of two adjacent nucleotides between the 3' hydroxy and the 5' phosphate. The ligation was performed in 10 µl reactions by using the appropriate molar ratio of plasmid vector to insert (1:3), 5 units of T_4 DNA ligase and 1X ligation buffer (250 mM Tris-HCl pH 7.6, 50 mM $MgCl_2$, 5 mM ATP, 5 mM DTT, 25% (w/v) polyethylene glycol-8000). The ligation mixture was incubated at 25°C-4°C for 16

hours. Recombinant plasmids were transformed into competent cells *E. coli* strain JM 109 (Promega) for amplifying the plasmids. The 2 µl of ligation mixture was added into 10 µl pre-warmed 65°C molecular grade water (Sigma Aldrich), mixed and incubated at 65°C for 20 minutes. After that, 200 µl of *E. coli* JM 109 competent cells was added with 10 µl of pre-warmed 65°C ligation mixture, mixed and incubated on ice for 30 minutes. The recombinant plasmids were introduced into the competent cells by heat-shock at 42°C for 50 seconds and put on ice immediately. Next, 400 µl of SOC medium was added into the cell mixture. After incubation at 37°C for 1 hour, the transformed cells were spread on LB agar plates, containing 100 µg/ml of ampicillin, 40 µl of 20 mg/ml X-gal and 100 µl of 0.1 M IPTG, and incubated overnight at 37°C. Transformants were differentiated by blue/white colonies.

4.2.2.4 Recombinant plasmid DNA extraction using modified CTAB method

The *E. coli* colonies containing recombinant plasmid (white colonies) were grown overnight in 3 ml LB broth containing 100 µg/ml of ampicillin. The 3 µl of cultured cells were centrifuged at 14,000 rpm for 1 minute. After discarding the supernatant, the pellet was resuspended in 200 µl of STET (8% sucrose, 0.1% TritonX-100, 50 mM Tris-HCl pH 8.0), added to 10 µl of 50 mg/ml lysozyme solution, mixed and incubated at 37°C for 10 minutes. The suspension was boiled for 45 seconds and centrifuged immediately at room temperature for 15 minutes. The pellet was removed using a toothpick and then 1/10 volume of 5% CTAB was added. After being centrifuged for 5 minutes, the pellet was resuspended in 300 µl of 1.2 M NaCl, vortexed, added with 5µl of 10 mg/ml RNase and incubated at 37°C for 15 minutes. After incubation, the mixture was added with an equal volume of chloroform-isoamyl alcohol, mixed, and then centrifuged for 5 minutes. The upper phase was transferred to a new tube, added with 1/10 volume of 5M NaCl, mixed, added equal volume of isopropanol and mixed. Following this, it was centrifuged at 14,000

rpm for 15 minutes, the pellet DNA was resuspended with 30 μl of molecular grade water (Sigma Aldrich). The recombinant plasmid was digested with *EcoR*I to roughly estimate size of the PCR fragments.

4.2.2.5 Detection of Microsatellite-Containing Clones Using PCR

PCR amplification with two vector primers (T7 and M13 reverse primers) and the nonbiotin-labelled $(AAAG)_6$ primer (S6) was used to identify the PCR product containing AAAG repeats. The PCR reactions were performed in a total volume of 25 μl with 1X premix PCR buffer (Sigma Aldrich) and 2 pmol of each primer. The reaction conditions were one cycle of 9 min at 95°C, 45 s at 60°C, 2 min at 72°C; 34 cycles of 45 s at 94°C, 45 s at 60°C and 2 min at 72°C; followed by 1 cycle of 5 min at 72°C. The products were visualized by agarose gel (1.5%) electrophoresis. Clones giving two (or more) bands were considered likely to contain a microsatellite and were PCR amplified with the vector primers T7 and M13 reverse using similar conditions to those given above. The PCR products were purified and cycle-sequenced using 5 pmol of T7 primer and BigDye Terminator V3.1 kit (Applied Biosystems) with procedures specified by the manufacturer. DNA sequences were determined using the Applied Biosystems 3130 DNA Analyzer. DNA sequences surrounding a microsatellite locus, termed the flanking regions, are generally conserved across individuals of the same species and sometime different species (Selkoe and Toonen 2006). Therefore, clones were chosen based on long flanking sequence with no sign of unambiguous motif, such as dinucleotide repeats. Therefore, primers for PCR amplification of each microsatellite locus were designed using the program VNTI (Invitrogen, Inc.) based on their flanking regions.

4.2.2.6 Microsatellite marker selection, labelling and multiplex PCR amplification

All primers designed for microsatellite analysis are listed in Appendix 3. As the markers were designed solely on an individual belonging to mitochondrial clade A3, it was necessary to test them with the preliminary group to examine whether they were applicable to the entire population. Each microsatellite marker was test-amplified with five individuals (JG01, SD01, Tb01, M01 and JI01) from the preliminary group. Successfully amplified markers with appropriate size (range between 100 to 600 bp) were chosen for further analysis.

Labelled Primers were ordered from Applied Biosystems. One primer from each pair was fluorescently end-labeled with 6-FAM™, VIC®, NED™ or PET® to distinguish each microsatellite loci. Multiplex amplifications were carried out in 25µl with 1X premix PCR buffer (Sigma Aldrich) and 0.25 ρmol of each primer. Two multiplexes were created according to the annealing temperatures. The PCR reaction conditions were: one cycle of 5 min at 95°C; 35 cycles of 1 min at 95°C, 1 min at 55 and 50°C for multiplex 1 and 2, respectively, and 1 min at 72°C; followed by 1 cycle of 10 min at 72°C. Fluorescently labeled fragments were run on an Applied Biosystems 3730 DNA Analyzer with the GeneScan LIZ600 size standard (Applied Biosystems).

4.2.2.7 Sizing and genotyping of microsatellite-containing amplicons

The fluorescent labelled fragments (referred here as amplicons) were subjected to microsatellite binning. This was done using GeneMapper® software version 4.0 (Applied Biosystems). Number of loci was calculated for each microsatellite marker. Polymorphism data were test for deviation from Hardy-Weinberg equilibrium and linkage disequilibrium. All calculations were performed using the program ARLEQUIN ver. 3.5 (Excoffier and Lischer 2010). A sequential Bonferroni correction were applied to correct for multiple comparisons (Rice 1989).

4.3 Results

4.3.1 Identification and quality control screening of microsatellite markers

Approximately 300 recombinant colonies were sequenced. Only 32 sequences (10%) contained at least six-uninterrupted microsatellite repeats and contained enough flanking sequence to allow design of primers. Ten out of fifteen microsatellite markers successfully amplified the preliminary group (data not shown). Eight of these microsatellite loci were pure tetranucleotide repeats, two loci (Tb72 and Tb240) were compound microsatellites (Table 11). These microsatellite markers were screened on the candidates from the SE group.

Polymorphism data were obtained by typing 40 individuals from Site 03 and 05 (n=20 each) in which corresponded to the two principal mtDNA lineage A1 and B1. Microsatellite markers were found to amplify reliably. All loci were variable within each site with the number of alleles per locus ranged from four to fifteen (Table 11). The shortest allele amplified was 122 basepairs (Tb49) and the longest allele was 577 basepairs. The observed and expected heterozygosities for Site 03 ranged from 0.45 to 1.00 and 0.54 to 0.94, respectively. The observed and expected heterozygosities for Site 05 ranged from 0.70 to 1.00 and from 0.53 to 0.93, respectively. Despite the significant deviations from Hardy-Weinberg equilibrium were detected in locus Tb72 [after sequential Bonferroni correction (Rice 1989)], there was no obvious evidence of null alleles. No evidence of linkage disequilibrium was found (after sequential Bonferroni correction).

4.3.2 Cross-species applications

Cross-species amplification of the multiplex PCRs was assessed by typing twenty individuals of *Tarentola delalandii* from a single site (Boca Cangrejo) on Tenerife, and 6 individuals of *Tarentola caboverdianus* from the island of São Vicente, Cape Verde. All ten microsatellite markers were successfully amplified in both species and were found to

be variable (3- 16 alleles in *T. delalandii*, and 3- 10 alleles in *T. caboverdianus*) (Table 12). Significant deviations from Hardy-Weinberg were observed in *T. delalandii* (locus Tb49), but none in *T. caboverdianus*. Significant linkage disequilibrium was detected for Tb192 and Tb35 in *T. caboverdianus* (data not shown).

4.4 Discussion

Total ten newly identified (AAAG)-repeated microsatellite loci were isolated from *T. boettgeri*. The percentage of recombinant clones was rather disappointing (10%) when compared to the rate of 16.7% in Gardner et al. (1999) and 20.3% in Bloor et al. (2006). The difference in enrichment efficiency, particularly lower percentage of recovery, may be due to the change in screening protocols. Alternatively, it may be due solely to nature of the genome of organism, itself. Note that we also discarded any clones containing recombinant plasmid with insertions smaller than 300 basepairs as they are unlikely to contain good flanking regions for primer design. However, the number of usable microsatellite loci (polymorphic and in HWE) obtain here are comparable to other microsatellite isolation experiments on lizards using the same protocols (e.g. Bloor et al. 2006; Suárez et al. 2008; Johansson et al. 2008b). Significant deviations from Hardy-Weinberg equilibrium detected in locus Tb72 of *T. boettgeri* could be due to the presence of null alleles or a Wahlund effect (Wahlund 1928). The latter explanation is quite feasible because of potential substructuring in the area: the two sites are close to the mtDNA contact zone. The observation of compound or interrupted tandem repeats is common phenomenon (Goldstein and Schlötterer 2001). The compound tetranucleotide repeats were also identified from *T. boettgeri* as in other reptiles, even when use different type of repeat and protocols (e.g. Bloor et al. 2008).

Table 11 Primers and descriptions of the ten microsatellites isolated from *Tarentola boettgeri*

Locus (accession number)	Primer**	Label	Repeat array in clone	Clone size (size range) bp	Site 03 (n = 20)			Site 05 (n = 20)		
					N_A	H_O	H_E	N_A	H_O	H_E
Tb48 (HM212426)	F48.2 (1) R48.1 (1)	6-FAM™	$(GAAA)_{23}$	179 (133 - 214)	15	0.75	0.90	11	1.00	0.88
Tb71 (HM212427)	F71.5 (1) R71.3 (1)	VIC®	$(GAAA)_{23}$	371 (324 - 395)	14	1.00	0.92	15	0.90	0.93
Tb72 (HM212428)	F72.1 (1) R72.2 (1)	NED™	$(GAAAGAGA)_4(GAAA)_{14}$	599 (488 - 567)	8	0.70	0.60	10	0.45*	0.77
Tb192 (HM212429)	F192.2 (1) R192.2 (1)	PET®	$(GAAA)_{17}$	399 (356 - 441)	14	0.95	0.93	16	0.85	0.93
Tb240 (HM212430)	F2401. (1) R240.3 (1)	6-FAM™	$(GAAA)_6(GA)_5(GAAA)_6$	530 (504 - 577)	11	0.85	0.89	13	0.95	0.88
Tb8 (HM212431)	F8.3 (2) R8.1 (2)	6-FAM™	$(GAAA)_{22}$	261 (210 - 280)	12	1.00	0.82	12	0.95	0.85
Tb35 (HM212432)	F35.1 (2) R35.1 (2)	VIC®	$(GAAA)_{23}$	424 (375 - 459)	14	1.00	0.93	14	0.85	0.92
Tb49 (HM212433)	F49.2 (2) R49.1 (2)	NED™	$(GAAA)_{19}$	160 (122 - 204)	4	0.70	0.53	5	0.70	0.54
Tb213 (HM212434)	F213.1 (2) R213.3 (2)	6-FAM™	$(GAAA)_{21}$	499 (459 - 540)	4	0.85	0.61	5	0.90	0.71
Tb234 (HM212435)	F234.2 (2) R234.3 (2)	PET®	$(GAAA)_{13}$	369 (331 - 392)	15	0.95	0.93	14	0.95	0.94

N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity. *Significant HWE deviation ($P < 0.05$). **PCR multiplex set in parentheses.

The variability detected at these microsatellite loci in *T. boettgeri* suggest they will provide an excellent tool for analyzing within-island nuclear DNA structuring relative to mtDNA structuring in both *T. boettgeri* and *T. delalandii,* which both show interesting patterns of within island evolution (Gübitz et al. 2000; Gübitz et al 2005). Successful cross-amplification in the Tenerife and Cape Verde *Tarentola* suggests that these loci are likely to be useful for the entire *Makariogecko* subgenus, which includes all the Western Canary and Cape Verde Island *Tarentola*, as well as a Moroccan species (Carranza et al. 2002).

Table 12 Polymorphism data for the multiplexed loci based on 20 individuals of *Tarentola delalandii* from Tenerife (Canary Islands) and 6 individuals of *Tarentola caboverdianus* from São Vicente (Cape Verde Islands).

	T. delalandii (n = 20)				*T. caboverdianus* (n = 6)			
Locus	N_A	Size range (bp)	H_O	H_E	N_A	Size range (bp)	H_O	H_E
Tb48	12	133 - 188	0.85	0.90	10	137 – 212	1.00	0.97
Tb71	15	329 - 398	1.00	0.92	8	326 – 384	1.00	0.92
Tb72	12	500 - 615	0.95	0.85	5	502 – 519	1.00	0.80
Tb192	13	357 - 423	0.95	0.91	6	368 – 396	0.50	0.89
Tb240	16	481 - 580	0.95	0.94	8	501 – 573	1.00	0.92
Tb8	11	210 - 303	0.90	0.80	4	211 – 252	0.50	0.45
Tb35	14	373 - 443	0.80	0.88	8	371 – 445	0.67	0.92
Tb49	3	137 - 204	1.00*	0.56	3	137 – 174	1.00	0.62
Tb213	8	459 - 547	0.85	0.84	7	464 – 550	0.67	0.77
Tb234	6	330 - 380	0.40	0.50	5	330 – 396	0.67	0.58

N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity.

Chapter 5 Variation in microsatellite DNA across the mtDNA contact zone

5.1 Introduction

Speciation occurs when reproductive isolation arises between differentiated gene pools (Noor 2002). Dobzhansky (1936) and Muller (1942) proposed that incidental genetic divergence in allopatry could create genetic incompatibilities, which resulted in substantial post-zygotic isolation. This hypothesis has been supported in several studies (reviewed in Orr and Presgraves 2000). Investigating genetic structure at the zone of secondary contact is, therefore, important for understanding the influence of genetic incompatibilities on maintaining species/population boundaries (Knowles and Richards 2005).

In previous chapters, I have demonstrated that *T. boettgeri* populations at the centre of the transect show a sharp transition in mtDNA lineage composition, but no corresponding change in morphology. Previous studies have demonstrated that nuclear genetic differentiation prevents successful reproduction among populations which show no clear morphological differentiation (e.g., Phillips et al. 2004; Furman et al. 2010). The aim of this study was to investigate the genetic structure of the gecko using nuclear gene markers.

Various nuclear markers have been employed to investigate patterns of genetic structure at contact zones among reptile populations, including single copy nuclear (scn) genes (e.g., Zarza et al. 2008; Okamoto and Hikita 2009) and microsatellites (e.g., Carlsson et al. 2004; Ğibbs et al. 2006; Godinho et al. 2008). At the beginning of this study, several scn genes, reported to successfully amplify in other reptiles [e.g., c-mos (Saint et al. 1998), MHC, GAPD (Dolman and Phillips 2004), RAG1 (Townsend et al. 2004), Phosducin (Bauer et al. 2007)] were tested with *T. boettgeri*. Only two of them (RAG1 and Phosducin) exhibited substitutions when populations from across the island were examined, and divergence was low (<1% segregating sites). In addition, the sequences

were indistinguishable between individuals from mtDNA lineages A and B. Thus, it was considered that they were not very suitable for this study.

There is evidence that analyses of several markers will provide better estimates of gene flow, natural selection, hybridization and linkage disequilibrium system compared with single-locus analyses (Simonsen et al. 1998; Kruuk et al. 1999b; Yang and Rannala 2010). This is because each genetic marker will have its own history, depending on fundamental processes, such as rates of recombination, mutation and selective constraints (Sunnucks 2000). It therefore appeared sensible to use microsatellite markers, for which several independent loci can be characterised together (as described in Chapter 4) and which show a higher evolutionary rate than other scn sequences. I aimed to use variation in microsatellite repeat number to infer introgression and gene flow across the contact zone. Influence of dispersal on microsatellite variation was also assessed. The results will also be useful to examine the hypothesis that the mtDNA pattern is due to the existence of two incipient species.

It is useful to consider how populations mix at a contact zone. MtDNA admixture at a contact zone can reflect either (i) simple temporary mixture of individuals from two populations, or (ii) interbreeding and formation of hybrids (Chow and Takeyama 2000; Nielsen et al. 2003; Durand et al. 2005). Simple admixture should leave its signature in the form of heterozygote deficiencies (Wahlund effect; Wahlund 1928) and linkage disequilibrium across loci that differ in allele frequency between populations (Nielsen et al. 2003), which are the result of strong barriers to gene flow (Barton 2000; Phillips et al. 2004). If the two populations interbreed, then no heterozygote deficiency should be present, even though genotypic disequilibria may be observed across loci when contact between populations is sufficiently recent (Durand et al. 2005).

In *T. boettgeri*, low mtDNA admixture and gene flow point to the hypothesis of two independent populations as a result of genetic incompatibilities. This would predict substantial deviation from Hardy-Weinberg and linkage disequilibrium at nuclear loci. More obviously, clear geographical structure of nuclear markers is expected, resulting from genetic divergence in allopatry. These patterns have been described by several other reptile studies in which divergence in allopatry has occurred (e.g., Carlsson et al. 2004; Howes et al. 2006; Demastes et al. 2007; Pedall et al. 2011). Under this hypothesis it would be expected to observe at least two main subpopulations comprising of individuals with differentiaed nuclear genotypes.

It is also possible that individuals from the two mtDNA lineages interbreed. If reproductive isolation is incomplete, introgressive hybridization between differentiated lineages may occur after secondary contact (Borge et al. 2005; Mallet 2005). Introgression rates can vary among loci (Payseur et al. 2004). In general, genes with alleles that reduce fitness in at least some environments or genetic backgrounds should be prevented from introgressing across the contact zones (Barton 2001). If there is strong selection acting on many loci distributed over the whole genome, all loci will form concordant clines in the change of allele frequency at the contact zone, which are maintained by a selection-dispersal balance (Gay et al. 2007). Nevertheless, neutral alleles (e.g., mtDNA) may pass easily across the zone unless generalized barriers, such as differential adaptations and genetic incompatibilities, are present (Grahame et al. 2006). At the contact zones where genetic incompatibilities are reported, concordant clines in the change of allele frequency among nuclear and mtDNA markers are usually observed (e.g., Alexandrino et al. 2005; Adams et al. 2006). Thus, in *T. boettgeri*, geographically concordant clines in allele frequency among all microsatellite and cytb markers would be expected under the genetic

incompatibility hypothesis. Levels of nuclear gene introgression and number of hybrid individuals would be expected to be low and observed only at the contact zone.

Alternatively, the spatial variation in microsatellites may be discordant with mtDNA. Spatial discordance between pattern of nuclear and mtDNA markers have been reported in many studies (e.g., Lu et al. 2001; Zarza et al. 2011). Apart from selection, several factors, such as sex-biased dispersal, and homoplasy, may explain this (Dowling and Secor 1997; Rieseberg 1998; Larmuseau et al. 2010). Population dynamics, spatial distribution and genetic structure are closely tied to patterns of dispersal, which is influenced by many characteristics of the individual, such as age or sex (Rivera et al. 2006). Sex-biased dispersal occurs when individuals of one sex move and breed at greater distance from the natal site than the other sex (Favre et al. 1997; Wolff and Plissner 1998). Sex-biased dispersal is a feature of the species' evolutionary ecology, since the dispersing sex is less structured (genetically) and should present a larger heterozygote deficit (Goudet et al. 2002). It also can generate variable introgression rates of loci across the genome leading to discordant geographical patterns among them (Barton and Hewitt 1989; Lyrholm et al. 1999; Castella et al. 2001). Therefore, it is necessary to investigate sex-biased dispersal in *T. boettgeri* to understand the role of dispersal in shaping genetic structure.

Male-biased dispersal is generally predicted in taxa with polygynous mating systems with a greater female investment in offspring, and female-biased dispersal in monogamous species where both parents share the cost of the offspring (Greenwood 1980; Dobson 1982; Wolff and Plissner 1998; Handley and Perrin 2007). Reptiles are mostly polygynous and male-biased dispersal has been demonstrated in lizards, such as the iguanidae (e.g., Doughty et al. 1994; Rassmann et al. 1997), scincidae (e.g., Olsson and Shine 2003), and anoles (e.g., Johansson et al. 2008a). There is no information on the

mating system of *Tarentola* geckos. Nevertheless, it was reported that geckos are generally gregarious, lack territoriality, and are likely to have a polygynandrous mating system (Todd 2005; LaDage et al. 2008). Moreover, rates of mtDNA gene flow suggest philopatry in female *T. boettgeri* (see Chapter 2). Hence, the initial expectation was that dispersal might be male-biased in *T. boettgeri*, and I aimed to test this using indirect methods that infer sex-specific dispersal rates from the spatial distribution of alleles or genotypes as described in Goudet et al. (2002).

If there is evidence of male-biased dispersal then discordance between geographical pattern of microsatellites and mtDNA is expected. This may also imply that the contact zone is maintained by endogenous selection and dispersal. Alternatively, geographical patterns of microsatellites may coincide with mtDNA. Such concordance is likely to arise only when populations have been reproductively separated from one another for a reasonably long period of time (Avis 1989; Avis and Ball 1990). This can be viewed as evidence that vicariance events have also played an important role in shaping the geographical distribution of nuclear DNA of *T. boettgeri*. In this case, the contact zone is likely to be shaped and maintained by endogenous selection (i.e., strong cyto-nuclear incompatibilities) rather than dispersal, which may be indicative of speciation. If this is the case, then it can be viewed as evidence that the contact zone is maintained by strong cyto-nuclear incompatibilities, which may be indicative of speciation.

5.2 Methodology

5.2.1 Amplification and evaluation of microsatellites

Ten microsatellite loci were characterized for all individuals captured from the transect. Primers and multiplex PCR for microsatellite amplifications were as described in Chapter 4. The software ARLEQUIN version 3.5 (Excoffier and Lischer 2010) was used

for exact testing of Hardy-Weinberg equilibrium and linkage disequilibrium for all localities and loci.

Microchecker version 2.2.3 (Dewoody et al. 2006) was used to detect three common scoring errors (null alleles, stuttering and large allele dropout [i.e. short allele dominance]). Microchecker identifies errors such as those in samples of panmictic populations of diploid organisms (van Oosterhout et al. 2004). Sequential Bonferroni corrections (Rice 1989) were applied wherever necessary. Any microsatellite locus that failed these tests was eliminated from the analysis.

5.2.2 Examination of sex-biased dispersal

Sex-biased dispersal was tested using the software FSTAT version 2.9.3 (Goudet 1995) to calculate gene diversity (H_S), F_{IS}, F_{ST}, mean assignment index (mAIC) and variance of the assignment index (vAIC) separately for each sex. The test was two-sided, i.e., the null hypothesis is that there is no sex-biased dispersal. Statistical significance of these indices was determined by 100 randomisations as implemented in the software. A bias in dispersal between the sexes should be reflected as statistically significant dissimilarity in the estimated parameters (Goudet et al. 2002).

5.2.3 Investigating population genetic structure

To infer population structure using microsatellite loci without defining a priori groups, a model-based clustering method, the admixture population ancestry model (allele frequency independent), implemented in STRUCTURE version 2.3.3 (Pritchard et al. 2000) was used. The number of population clusters (K) was estimated using two methods: (i) average values of likelihood scores [L(K)], and (ii) the second order rate of change of the likelihood (ΔK) as described in Evanno et al. (2005). Likelihood scores [Ln P(D)] were obtained from three Bayesian clustering replicates with k ranging from 1 to 5 (assuming the number of populations was three, i.e., populations with mtDNA clade A, mtDNA clade

B, and hybrid populations). Each replicate was run for 10^6 generations and the first 10^6 generations discarded as burn-in. Individuals were assigned to a population on the basis of their genotypes (Pritchard et al. 2000).

5.2.4 Studying pattern of microsatellite gene flow

The program MIGRATE version 3.1.6 (Beerli 2008) was used to estimate long-term gene flow across the transect, similar to the mtDNA study (Chapter 2, section 2.2.7). In this study, microsatellite evolution was modelled as a continuous Brownian process. Parameter distributions were estimated using the maximum likelihood coalescent approach (Beerli and Felsenstein 1999; 2001). Following a burn-in of 10^4 iterations, both runs were carried out with 5×10^5 and 5×10^6 genealogies for short- and long chains, respectively which were sampled every 100 iterations. The program was run twice and results were generally stable.

5.3 Results

5.3.1 microsatellite variation and sex-biased dispersal

A total of 390 individuals were successfully genotyped, of which 222 were female and 168 were male. All loci were variable within each site with the number of alleles per locus ranged from four to twenty-two (Table 13). Significant deviations from Hardy-Weinberg equilibrium were detected mostly at the centre (site 01, where three mtDNA lineages were found), and at both ends of the transect (sites 02 and 11; see Table 14). Several pairs of loci demonstrated evidence of linkage disequilibrium (after sequential Bonferroni correction). Significant disequilibria between pairs of loci is particularly high at sites 02 and 12, which are located at the southern end of the transect (Figure 23). Linkage disequilibrium was also observed at sites 05, 06, 10 and 11, which are located at the northern end of the transect (Figure 23).

Microchecker did not reveal evidence of null alleles, stuttering or large allele dropout observed in any loci (data not shown). Microsatellite locus Tb49 demonstrated high homozygosity (more than 50%), which might be due to non-random mating (Palo et al. 1995; Wattier et al. 1998). Nevertheless, adding or removing this locus did not have any impact on subsequent analyses. Therefore, reported results are based on all ten microsatellite loci.

Results obtained from tests for sex-biased dispersal are shown in Table 15. The sex with greater dispersal should exhibit higher within-group gene diversity (H_S), heterozygote deficit (F_{IS}) and variance of assignment index value (vAIC), while the philopatric sex should exhibit higher among-groups genetic variance (F_{ST}) and average assignment index value (mAIC) (Goudet et al. 2002). Thus, the results tentatively suggest that males may have a higher dispersal rate than females. However, there were no significant differences between male and female in any of these indices, so this hypothesis is not statistically supported.

5.3.2 Population structure inferred from microsatellite data

Average values of likelihood scores [L (K)] were lowest for one [k = 1, L(K) = -19200.83] but higher for two clusters [k = 2, L (K) = -18240.57] (Figure 24). However, the L (K) for k =3 (-18257.60) is quite similar to that of k = 2. Nevertheless, the ΔK show a clear peak at k = 2 (Figure 25), supporting two main genetic groups.

Table 13 Number of microsatellite alleles per locus per site.

Microsatellite Locus	Site													
	01	02	03	04	05	06	07	08	09	10	11	12	13	14
Tb48	12	16	12	18	12	12	14	11	11	12	16	10	10	10
Tb71	7	16	17	18	19	16	17	16	19	16	17	16	14	10
Tb72	12	13	9	9	12	9	16	5	6	8	11	7	6	9
Tb192	13	19	17	16	18	17	17	12	15	15	18	17	13	15
Tb240	13	10	13	11	14	15	15	14	10	14	16	10	10	11
Tb8	12	16	13	13	13	13	13	13	15	13	16	12	11	11
Tb35	9	14	14	17	15	19	20	15	16	14	22	17	10	13
Tb49	16	8	4	5	5	4	4	10	8	7	6	8	9	4
Tb213	15	15	4	12	5	11	13	14	17	11	13	15	6	11
Tb234	15	21	15	13	15	19	16	12	17	15	21	13	15	12

Table 14 A summary of the exact testing for Hardy-Weinberg disequilibrium for each site.

Site / Locus	01		02		03		04		05		06		07		08		09		10		11		12		13		14	
	H_O	H_E	H_O	H_E	H_O	H_E	H_O	H_E	H_O	H_E	H_O	H_E	H_O	H_E	H_O	H_E	H_O	H_E	H_O	H_E	H_O	H_E	H_O	H_E	H_O	H_E	H_O	H_E
Tb48	1.00*	0.87	0.86	0.91	0.77	0.87	0.93	0.93	0.90	0.87	0.67	0.89	0.91	0.87	0.69	0.70	0.83	0.72	0.93	0.81	1.00	0.91	0.96	0.80	0.82	0.82	0.62	0.83
Tb71	0.29	0.37	0.66*	0.90	0.93	0.90	0.93	0.93	0.87	0.94	0.97	0.93	0.88	0.94	0.93	0.93	0.80	0.93	0.80	0.92	0.70*	0.92	0.86	0.94	0.76	0.94	1.00	0.90
Tb72	0.94*	0.82	0.45*	0.62	0.59	0.56	0.70*	0.76	0.53*	0.73	0.70	0.66	0.63*	0.78	0.71	0.64	0.53	0.62	0.60	0.66	0.87	0.84	0.44	0.54	0.53	0.63	0.85	0.82
Tb192	0.65	0.85	0.62*	0.95	0.96	0.94	0.96	0.91	0.84	0.93	0.90	0.94	0.84	0.93	1.00	0.91	0.83	0.92	0.90	0.92	0.77	0.93	0.92	0.91	1.00	0.93	0.85	0.94
Tb240	0.90*	0.84	0.90	0.88	0.83	0.88	0.87	0.87	0.90	0.87	0.87	0.87	0.91	0.91	0.69*	0.90	0.80	0.84	0.80	0.86	0.80	0.88	0.64	0.85	0.53*	0.80	0.92	0.89
Tb8	0.97	0.90	0.97	0.90	0.97	0.85	1.00	0.79	0.93	0.80	0.90	0.83	0.94	0.90	0.96	0.79	0.97	0.83	0.96	0.79	0.87*	0.89	0.68	0.80	0.88	0.79	0.92	0.82
Tb35	0.42*	0.79	0.77	0.87	0.83	0.90	0.93	0.90	0.90	0.90	0.34*	0.95	0.66*	0.94	0.76	0.89	0.63	0.88	0.75	0.89	0.50*	0.95	0.52*	0.95	0.76	0.85	1.00	0.94
Tb49	0.94*	0.93	0.77	0.65	0.60	0.48	0.83	0.60	0.80	0.59	0.10	0.13	0.31	0.28	0.86	0.68	0.90	0.70	0.87	0.64	0.58	0.47	0.83	0.69	0.71	0.64	0.50	0.43
Tb213	0.84	0.92	0.90*	0.86	0.87	0.60	0.83*	0.84	0.93	0.72	0.76	0.76	0.78	0.79	0.54*	0.89	0.68*	0.90	0.89	0.82	0.67*	0.82	0.67	0.86	0.71	0.75	1.00	0.90
Tb234	0.67*	0.90	0.90	0.94	0.97	0.92	0.97	0.91	0.97	0.93	0.86	0.93	0.94	0.93	0.74	0.89	0.76	0.92	0.93	0.92	0.65*	0.96	0.59*	0.92	0.88	0.93	0.83	0.91

The test of HWE deviation is done using modified version of the Markov-chain random walk algorithm described in Guo and Thompson (1992).

H_O, observed heterozygosity; H_E, expected heterozygosity. *Significant HWE deviation ($P < 0.05$) after Bonferroni correction (Rice, 1989).

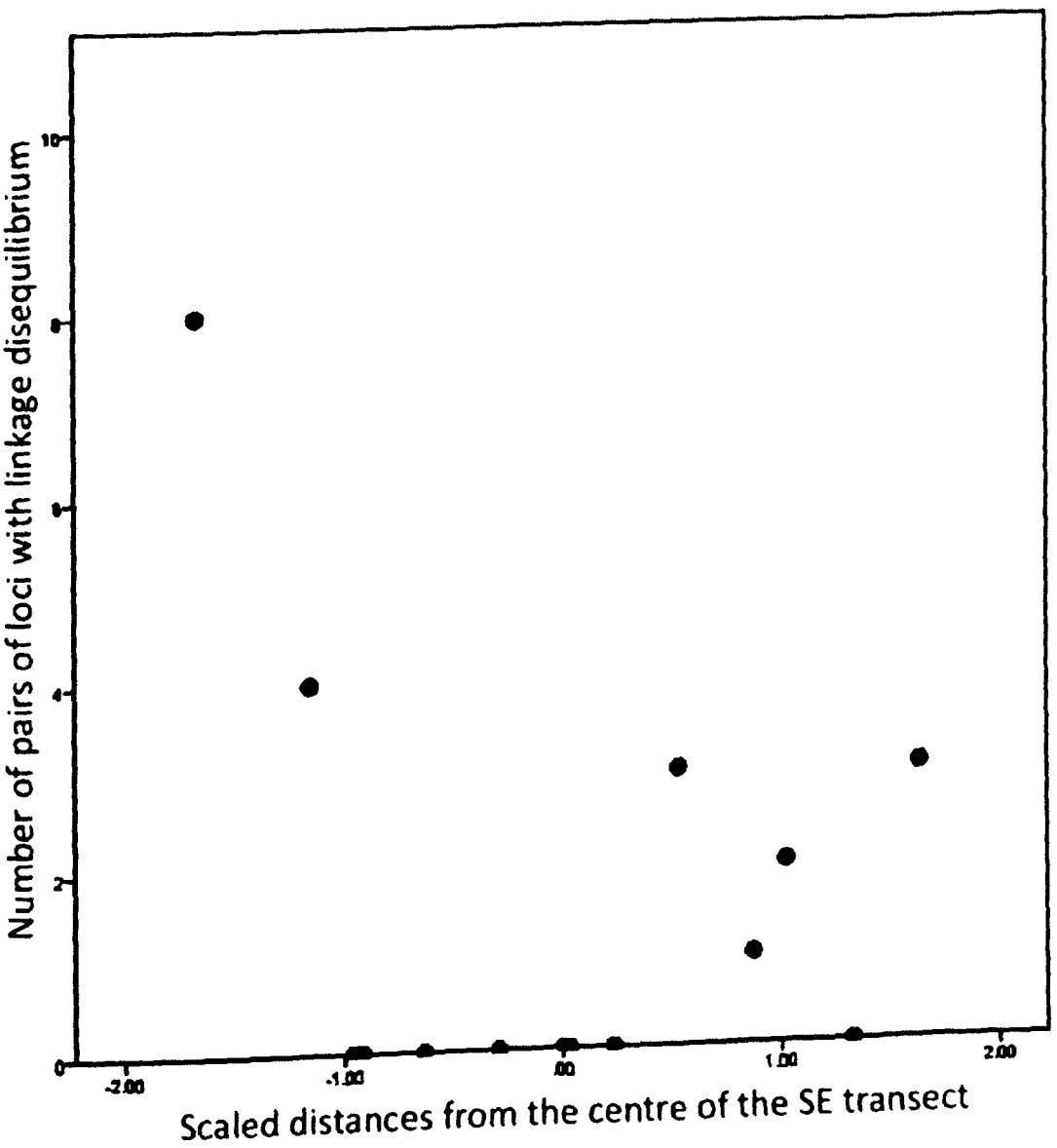


Figure 23. Summary of linkage disequilibrium analysis between pairs of microsatellite loci for each population along the SE transect. Proportion of locus pairs in significant linkage disequilibrium is shown against scaled distances from the centre of the transect. (Significant level = 0.05)

Table 15 Summary results for sex-biased dispersal for fourteen localities within the SE transect. Deviation from Hardy-Weinberg expectations (F_{IS}), F_{ST}, gene diversity (H_S) mean assignment index (mAIC) and variance of mean assignment index (vAIC) for females (F) and males (M) are shown. *P*-values are from two-tailed tests.

	F_{IS}	F_{ST}	H_S	mAIC	vAIC
F	0.039	0.044	0.821	0.134	13.171
M	0.044	0.033	0.828	-0.177	16.566
p-value	0.870	0.090	0.320	0.490	0.140

A geographically structured cluster of microsatellite genotypes is shown in Figure 26. Individuals were mostly assigned to cluster 1 (see Table 16). Within this cluster, some microgeographical substructuring is also apparent. Individuals assigned to cluster 2 are found mainly within sites 01, 02 and 11, which are located at the central and extreme ends of the transect. Genotype diversities between clusters are low (*p*-distance = 0.092). There was no clear evidence of parental and hybrid forms. Few individuals showed signs of co-ancestry, except for a few individuals admixed between clusters 1 and 2 mainly at sites 04, 07 and 11 (Figure 26).

Additional analysis was carried out to clarify how microsatellite genotypes were structured outside the SE transect using individuals sampled from site SA, Tb, SA, JG, M and JI. The results show that most individuals were assigned to cluster 1. Individuals from site JG and JI showed admixture between cluster 1 and 2 (see Appendix 4).

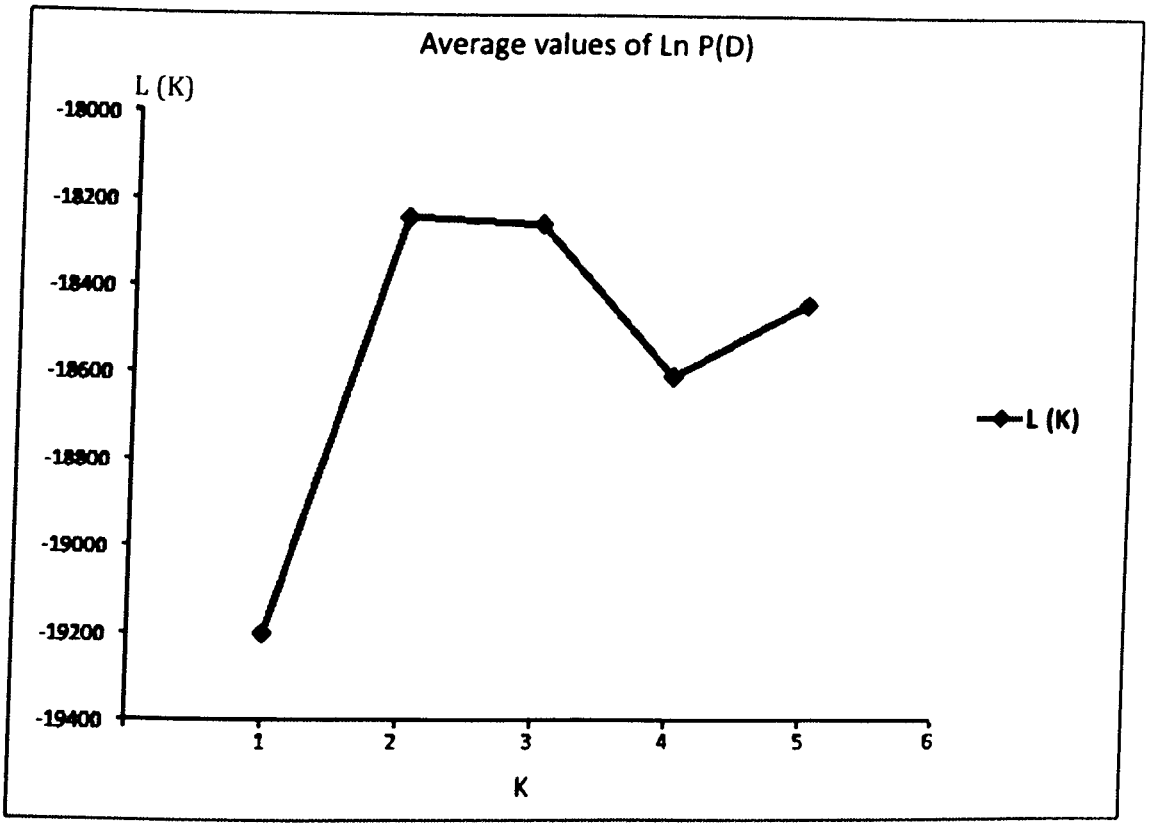


Figure 24. Graphical summary of mean values of LnP(D) [L(K)] (vertical axis) over 3 runs for each *k* value, where *k* is the number of population clusters.

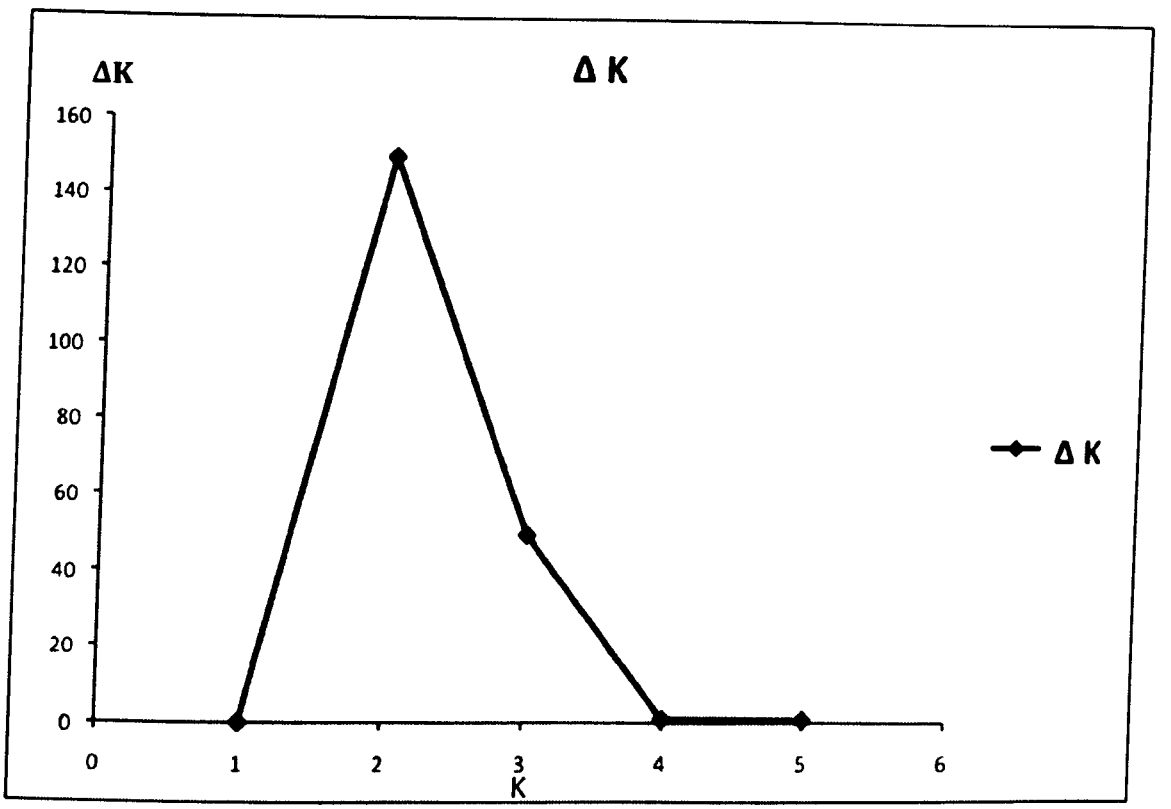


Figure 25. Graphical summary of the second order rate of change (ΔK) of the likelihood against number of cluster (K).

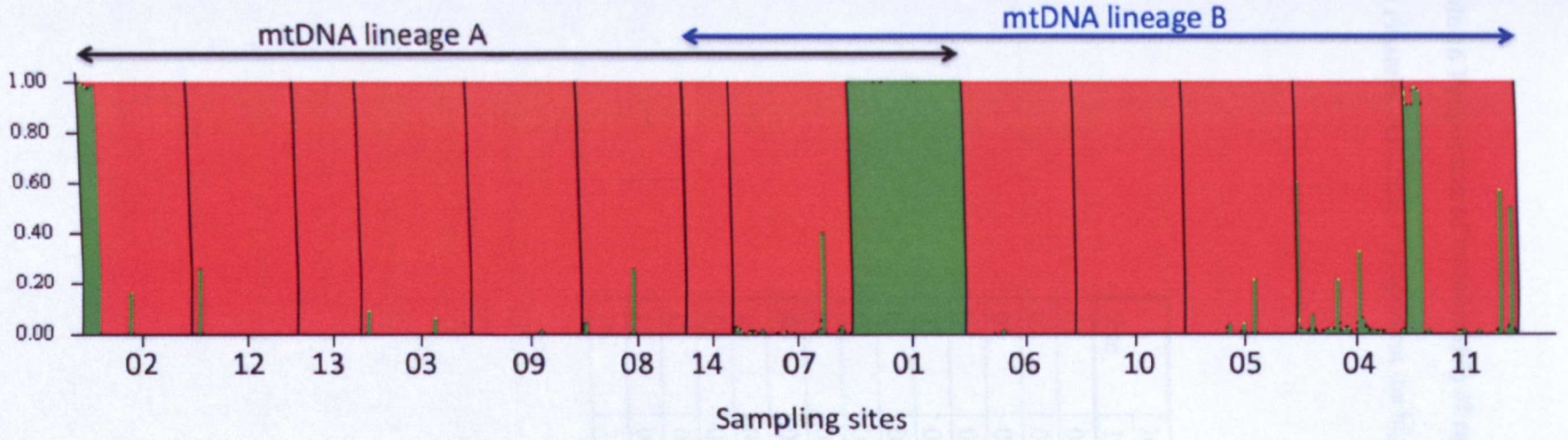


Figure 26. Assessment of population structure based on ten microsatellite loci and assuming two populations (k = 2). The y-axis represents proportions of the multilocus genotype in each sampling site that is attributable to co-ancestry with cluster 1 (red) and 2 (green). Individuals are grouped along the x-axis according to their sampling sites. (Inset: geographical map of sampling sites, detailed in Figure 11)

Table 16 Proportion of membership of each pre-defined 14 populations in each of the two clusters. Asterisks (*) represent the highest proportion of membership.

Site	Inferred Clusters	
	1	2
01	0.001	0.999*
02	0.827*	0.173
03	0.993*	0.007
04	0.945*	0.055
05	0.987*	0.013
06	0.997*	0.003
07	0.978*	0.022
08	0.987*	0.013
09	0.998*	0.002
10	0.998*	0.002
11	0.805*	0.195
12	0.988*	0.012
13	0.998*	0.002
14	1.000*	0.000

5.3.3 Pattern of gene flow across the transect

Pairwise migration rates against pairwise geographical distances are shown in Figure 27. Gene flow is not associated with either geographical distances or mtDNA lineage.

Rates of gene flow between sampling sites are generally similar (Table 17). The majority of individuals from the north of the contact zone correspond to mtDNA lineage B1while most individuals from the south of the contact zone correspond to mtDNA lineage A1. Rates of gene flow from south to north (1.02) are slightly lower than from north to south (1.07; Figure 28). However, it conflicts with the pattern of mtDNA gene flow, which suggested higher rates of gene flow from the south to the north of the transect.

5.4 Discussion

5.4.1 Influence of gene flow and dispersal on population structure based on microsatellites

The absence of prominent differentiation between populations north and south of the mtDNA contact zone implied that historical isolation has played little or no role in the formation and maintenance of the observed nuclear genetic structure in *T. boettgeri*. Hence, what are the factors underpinning nuclear genetic variation in this study area? The dual influences of gene flow and dispersal on shaping and maintaining pattern of genetic structuring in reptiles are known to be important (e.g., Ciofi and Bruford 1999; Ujvari et al. 2008). It has been suggested that genetic structuring could be induced solely by limited dispersal ability of individuals that prevent genetic exchanges throughout a given area (Slatkin 1993). When dispersal and gene flow are highly restricted, the species usually shows strong geographical structure (e.g., Prosser et al. 1999; Dubey and Shine 2010). In contrast, in the absence of strong barriers to gene flow, the difference in allele frequency is expected to fade away with time under the effect of dispersal (Gay et al. 2007).

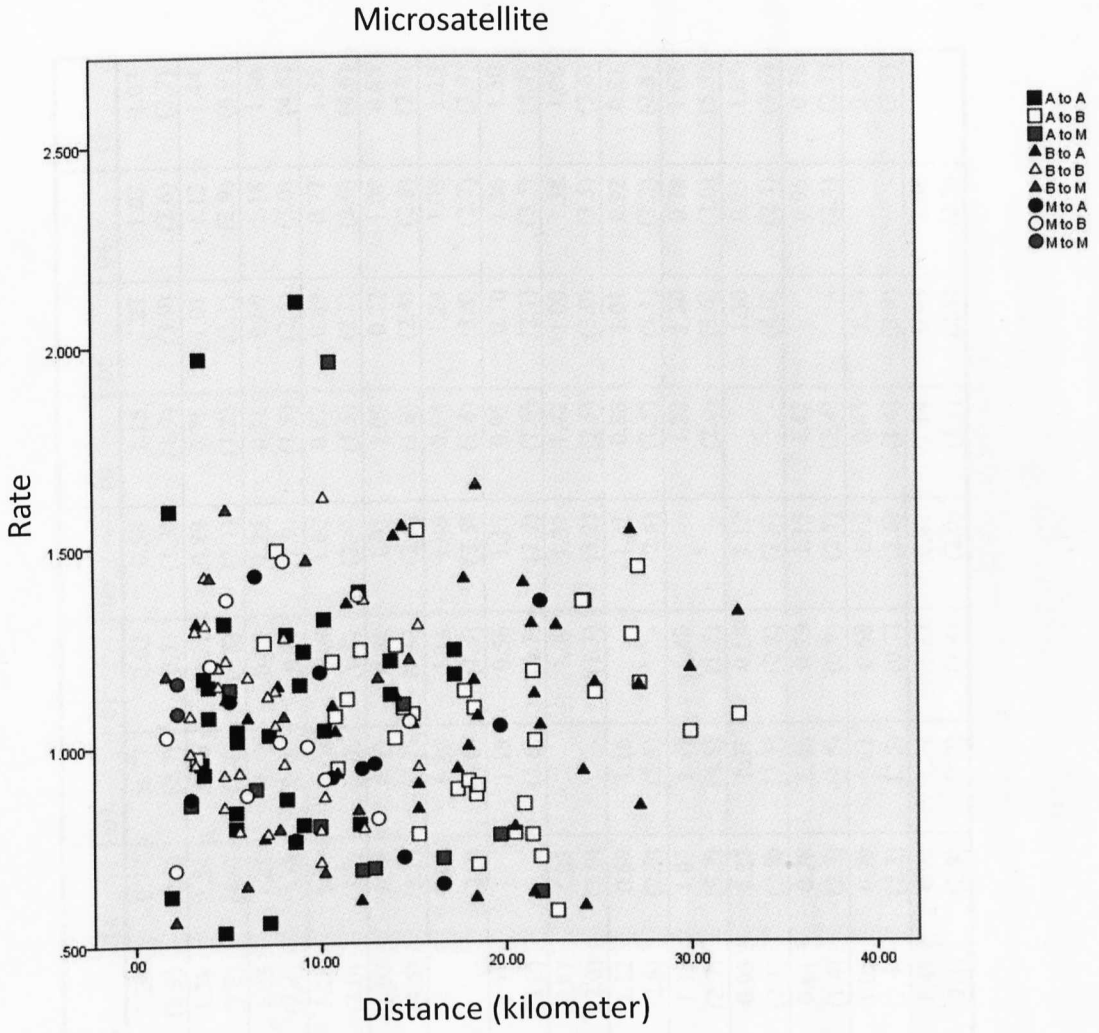


Figure 27. Scatter plots of migration rates (based on microsatellites) and geographical distances between sampling sites. Squares, triangles and circles represent migration rates from sites comprising mtDNA lineages A, B and sites with both mtDNA lineages (site 01 and 07 only). Pearson correlations (r) between migration rates and distances are: $r_{A\ to\ A}$ = 0.101 (p = 0.596), $r_{A\ to\ B}$ = -0.079 (p = 0.649), $r_{A\ to\ M}$ = -0.274 (p = 0.388), $r_{B\ to\ A}$ = 0.055 (p = 0.744), $r_{B\ to\ B}$ = -0.066 (p = 0.733), $r_{B\ to\ M}$ = 0.020 (p = 0.950), $r_{M\ to\ A}$ = 0.000 (p = 0.999), $r_{M\ to\ B}$ = 0.075 (p = 0.816).

Table 17 Migration rates of microsatellites between sampling sites. Sites are arranged according to their position on the transect, from south to north. Numbers of migrants per generation are in brackets.

To site / From site	02	12	13	03	09	08	14	07	01	06	10	05	04	11
02	-	0.83 (2.0)	1.04 (2.9)	1.24 (2.5)	1.21 (2.7)	1.24 (2.9)	0.77 (2.3)	0.77 (2.3)	0.62 (0.7)	0.58 (1.3)	1.15 (1.9)	1.27 (3.9)	1.03 (2.6)	1.07 (3.7)
12	0.79 (1.8)	-	1.01 (2.8)	1.15 (2.3)	2.11 (4.7)	1.38 (3.2)	1.54 (4.6)	1.10 (3.4)	0.71 (0.8)	0.89 (2.1)	0.71 (1.2)	1.01 (3.1)	1.13 (2.9)	1.44 (4.9)
13	1.32 (3.1)	1.04 (2.5)	-	1.59 (3.2)	0.93 (2.1)	1.03 (2.4)	1.21 (3.6)	0.80 (2.4)	0.68 (0.8)	1.25 (2.9)	0.91 (1.5)	0.69 (2.1)	1.18 (3.0)	1.36 (4.6)
03	0.80 (1.9)	1.07 (2.6)	0.62 (1.7)	-	1.31 (2.9)	1.28 (3.0)	1.12 (3.3)	1.96 (6.0)	0.69 (0.8)	1.02 (2.4)	0.87 (1.5)	0.89 (2.7)	0.77 (2.0)	1.35 (4.6)
09	1.13 (2.6)	1.15 (2.8)	1.17 (3.3)	0.53 (1.1)	-	1.97 (4.5)	1.26 (3.7)	0.89 (2.7)	0.76 (0.9)	1.07 (2.5)	1.09 (1.8)	0.77 (2.4)	1.09 (2.8)	0.85 (2.9)
08	1.17 (2.7)	0.80 (1.9)	0.55 (1.6)	0.87 (1.8)	0.96 (2.1)	-	0.97 (2.9)	0.86 (2.6)	1.14 (1.3)	1.49 (3.5)	0.94 (1.6)	1.24 (3.8)	1.08 (2.7)	1.13 (3.9)
14	0.79 (1.8)	0.90 (2.2)	1.10 (3.1)	1.35 (2.8)	0.77 (1.7)	1.31 (3.0)	-	1.18 (3.6)	0.56 (0.7)	1.17 (2.7)	0.95 (1.6)	0.70 (2.1)	1.36 (3.5)	1.30 (4.4)
07	1.04 (2.4)	0.72 (1.7)	1.19 (3.3)	0.92 (1.9)	1.43 (3.2)	0.87 (2.0)	1.03 (3.0)	-	1.09 (1.3)	1.37 (3.2)	1.47 (2.5)	1.00 (3.0)	1.38 (3.5)	1.06 (3.6)
01	1.36 (3.2)	0.65 (1.5)	0.94 (2.6)	0.95 (1.9)	0.76 (1.7)	1.12 (2.6)	0.69 (2.0)	1.16 (3.5)	-	1.21 (2.8)	0.88 (1.5)	1.01 (3.1)	0.92 (2.3)	0.81 (2.8)
06	1.29 (3.0)	0.94 (2.2)	1.12 (3.1)	1.52 (3.1)	1.03 (2.3)	1.15 (2.7)	1.07 (3.2)	1.59 (4.8)	1.42 (1.7)	-	1.22 (2.0)	1.20 (3.6)	0.78 (2.0)	1.62 (5.5)
10	0.84 (2.0)	1.04 (2.5)	0.99 (2.8)	0.61 (1.2)	1.55 (3.5)	0.93 (2.1)	1.27 (3.8)	1.07 (3.3)	0.64 (0.8)	1.12 (2.6)	-	1.30 (4.0)	0.93 (2.4)	1.05 (3.6)
05	1.53 (3.6)	1.12 (2.7)	1.07 (3.0)	1.65 (3.3)	0.84 (1.9)	0.61 (1.4)	0.78 (2.3)	1.46 (4.5)	0.79 (0.9)	1.15 (2.7)	1.42 (2.4)	-	0.95 (2.4)	0.78 (2.7)
04	1.19 (2.8)	1.15 (2.8)	0.62 (1.7)	1.30 (2.6)	1.16 (2.6)	1.05 (2.4)	0.79 (2.3)	0.83 (2.5)	0.68 (0.7)	1.12 (2.6)	0.85 (1.4)	1.29 (3.9)	-	0.98 (3.3)
11	1.33 (3.1)	1.15 (2.7)	0.59 (1.6)	0.93 (1.9)	1.40 (3.1)	1.41 (3.3)	0.94 (2.8)	1.21 (3.7)	1.17 (1.4)	0.87 (2.0)	1.14 (1.9)	0.93 (2.8)	1.08 (2.7)	-

Figure 28. Diagram showing directions of gene flow across the mtDNA contact zone. The yellow broken line shows the centre of the zone. White arrows indicate bidirectional gene flow across the contact zone. Numbers in parenthesis are average rates of gene flow (μ) from south (red) and north (light blue) of the contact zone. MtDNA admixture sites are within the green circle. Red arrows represent gene flow from south, while light blue arrows represent gene flow from the north.

If gene flow among sampling sites is not highly restricted, this would have lead to an isolation-by-distance pattern, in which neighbouring populations show closer genetic affinities than do geographically distant populations (Wright 1943). In this study, there was no correlation between genetic structure and distances (see Figure 27). It suggests 1) dispersal is highly limited or 2) the study area did not cover the maximum range of dispersal of the species (Monsen and Blouin 2003). There is no evidence supporting limited dispersal in *T. boettgeri*. Therefore, this hypothesis is rejected. Despite the fact that there are no studies that have provided direct measures of dispersal distances in *Tarentola* geckos, short-dispersing distances and low rates of dispersal have been reported in other geckos. A study of the gecko, *Gekko japonicus*, demonstrated limited long-distance dispersal was a likely explanation of the population genetic variation in this species (Toda et al. 2003). Evidence of low dispersal has been reported from mark-captured studies of *Hemidactylus turcicus*, which also demonstrated that the movement was less than 6 km (Selcer 1986). In *T. boettgeri*, migration rates estimates (estimated between 1.0-3.9 immigrants/generation) are much lower compared to species such as the garter snake, *Thamnophis sirtalis sirtalis* (2.7-37.6 migrants/generation: Bittner and King 2003), and the túngara frog, *Physalaemus pustulosus* (16 migrants/generation: Lampert et al. 2003). However, these species are clearly highly vagile. Instead, estimates are comparable with species known to disperse only short distances, such as the white-toothed shrew *Crocidura russula* (1.5 migrants/generation; Favre et al. 1997). This study supports the idea that dispersal in *T. boettgerii* is low and that this may have influenced the nuclear genetic structure.

The results suggested gene flow among site 01, 02 and 03 led to greater nuclear background similarity at these sites. However, based on their locations, direct migration among these sites is less likely. Nevertheless, human-induced migration may occur (see

previous discussion). Neither explanation is possible for mtDNA. That is because no B1 individuals are found at site 02, despite the results suggesting migration from site 01 and 11. A possible hypothesis is that site 02 and 11 may have received some gene pool from other populations outside the SE transect, which share similar nuclear DNA background to site 01. Thus, additional intensive sampling and microsatellite studies will be necessary to examine this further. Nevertheless, this does not explain why nuclear composition of site 01 differs from other sites. It may be a result of interbreeding among individuals from three mtDNA lineages (A1, B1 and B2). Nevertheless individuals containing mtDNA lineage A1 and B1 are not differentiated in terms of microsatellite DNA. Although two individuals belonging to mtDNA lineage B2 are also observed at site 06, the nuclear genotypes are not similar to site 01. Hence the explanation of "contamination" by individuals from the B2 mtDNA lineage also appears unlikely.

5.4.2 Lack of correlation between spatial patterns of microsatellite and mtDNA variation

The analysis of nuclear genetic structure revealed two subpopulations with a substantial degrees of genetic differentiation. Yet, this does not reflect mtDNA phylogeographical groupings. Generally, nuclear-mtDNA spatial discordance is explained as being due to (1) size homoplasy (Larmuseau et al. 2010), or (2) sex-biased dispersal (e.g., Gay et al. 2007; Lukoschek et al. 2008). It was proposed that size homoplasy may reduce the signal of differentiation detected by the microsatellite markers, thus, causing dissociation between spatial patterns of mtDNA and nuclear DNA (Estoup et al. 1995; Liepelt et al. 2001). Nevertheless, in this study, the allelic variation and wide size range of microsatellite markers indicated substaintial nuclear DNA divergence, thus, homoplasy is unlikely to explain this (see Appendix 5). In addition, simulation studies suggest that size

homoplasy will have less effect on estimates of population differentiation than gene migration (Estoup et al. 2002).

A lack of correlation between mtDNA phylogeography and nuclear DNA genetic data is often attributed to differences in the levels of male and female gene flow (Waits et al. 2000). This explanation is not well supported as the tests revealed that sex-biased dispersal is not significant. Nevertheless, the results do not fully reject a minor role of sex-biased dispersal on nuclear-mtDNA discordance (see section 5.4.4).

Apart from the possibilities mentioned above, it has been reported that nuclear gene flow among populations can account for discordance irrespective of sex-biased dispersal (e.g., García-París et al., 2003; Gonzalez and Zardoya 2007). In *T. boettgeri*, the pattern of nuclear gene flow clearly differs from that of mtDNA. This would be expected to provide different spatial patterns. However, the reasons for these differences in patterns of gene flow between nuclear and mtDNA are difficult to explain using the information obtained so far.

5.4.3 Deviations from Hardy-Weinberg equilibrium and linkage disequilibrium

Deviations from Hardy-Weinberg equilibrium (HWE) are observed in eleven out of fourteen sampling sites, mostly at sites 01, 02 and 11. This could be a result of (1) the Wahlund effect (Wahlund 1928) and/or (2) dispersal and selection against particular heterozygotes (MacCullum et al. 1998). The Wahlund effect has been extensively used to explain deviation from HWE in contact zone studies (e.g., Daguin et al. 2001; Pereira and Wake 2009). Nevertheless, this explanation is not appropriate here as there is no evidence of a nuclear DNA contact zone.

In species with limited dispersal, inbreeding leads to deviation from HWE (Gutiérrez-Rodríguez and Lasker 2004), for example, the geckos, *Oedura reticulata* and *Gehyra variegata*, which tend not to disperse far from their natal sites (Sarre et al. 1995;

Hoehn et al. 2007). If there is selection against hybrids, then deviation from HWE should be observed at many loci (Griebeler et al. 2006; Arias et al. 2008; Maletzky et al. 2010). In this study, low migration rates suggested low dispersal. Despite a lack of obvious environmental variation, endogenous selections may be operating (Phillips et al. 2004). A lack of hybrid genotypes in this study also supports this. Therefore, a combination of dispersal and selection is the most likely explanation of deviation from HWE, particularly at site 01.

Linkage disequilibrium (LD) is not always due to close proximity of markers on the chromosome, but can occur due to admixture or migration (Lewontin and Hartl 1991). At the location where two genotypes meet, the presence of LD, coupled with deviation from HWE, suggests that random mating has not yet been established and admixture/hybridization of subpopulations is recent (Waits et al. 2000; Redenbach and Taylor 2003; Rubidge and Taylor 2004). LD was detected at half the sites. At some of these sites (e.g., 02 and 11), evidence of deviation from HWE was also observed. Therefore, it is reasonable to conclude that gene flow among subpopulations has occured quite recently and has not yet reached equilibrium. In summary, genetic structure in *T. boettgeri* appears to be a result of non-random mating, low dispersal and/or unknown endogenous factors.

5.4.4 Evidence of sex-biased dispersal?

During fieldwork, male geckos were often found with one female under the same rock. Nevertheless, at some sample sites where geckos were very abundant, two females were often observed with one male (personal observation). These observations are similar to *Hoplodactylus maculatus* gecko, which is viewed as evidence of a polygynous species (Todd 2005). Male-biased dispersal is expected in polygynous species.

In a study of the large treeshrew, *Tupaia tana*, it was demonstrated that sex-biased dispersal can also be inferred from different migration rates between microsatellite and mt loci (Munshi-South 2008). If migration rates based on microsatellites are lower than those of mtDNA, then females disperse more than males. On the other hand, if mtDNA migration rates are lower, then females philopatry is inferred. The overall number of migrants estimated (using MIGRATE) from microsatellites (~2.6 migrants/generation) was more than three time higher than for mtDNA-based estimates (~0.7 migrants/generation). The substantially higher migration rates for microsatellites suggested that historical gene flow in *T. boettgeri* has been male-biased, which might contribute to the mtDNA-nuclear discordance. Nevertheless, male-biased sex dispersal was not detected here. This is perhaps not surprising. First, simulation studies have shown that the power of the tests is low when the bias is lower than 80:20, i.e., at least 80% of males disperse and only 20% of females disperse (Goudet et al. 2002). It has also been suggested that when migration is low, such as in *T. boettgeri,* immigrants are very rare and may thus pass undetected (Goudet et al. 2002; Vitalis 2002). Second, it is likely that females *T. boettgeri* are not entirely philopatric. In polygamous species, the female takes responsibility for acquiring and defending the territory for nesting and feeding the offspring and, therefore, benefits more from philopatry (Perrin and Mazalov 1999). Nevertheless, in some species, females also disperse but less frequently and over smaller distance than males, and thus weaken the sex-bias in dispersal (e.g., Mossman and Waser 1999; Lampert et al. 2003). In *T. boettgeri*, gene diversity (H_S) values are almost equal between males and females (Table 15) suggesting that dispersal may occur equally in both sexes.

In conclusion, the nuclear genetic variation in *T. boettgeri* clearly differs from the phylogeographical pattern but it is not entirely clear as to why this is so. Low dispersal

seems to explain some of the nuclear genetic structure, with dispersal rates appearing to be similar to those in quite philopatric species.

Chapter 6 Conclusion and General Discussion

6.1 No evidence of reproductive isolation or speciation among populations from different mtDNA lineages

Speciation arises as reproductive isolation evolves between diverging populations (Orr and Presgraves 2000). In *T. boettgeri*, persistence of spatial pattern of mtDNA led to the hypothesis of reproductive isolation between mtDNA lineages. Under this hypothesis, the following predictions were made (1) concordant spatial patterns of mtDNA and microsatellites, (2) low rate of gene flow across the contact zone, and (3) evidence of mechanical barriers to gene flow and reproduction. However, this is not supported by my analyses of microsatellites and morphology.

There are reasons to expect concordance of nuclear and mtDNA lineages. Several mitochondria genes encoding functional units are tightly linked with genes encoded in nuclear genomes. This suggests potential for co-evolution between mitochondria and nuclear genomes (Schmidt et al. 2001; Gershoni et al. 2009). Spatial patterns of mtDNA could be explained as result of selection against individuals with different mtDNA lineages and nuclear genomes because of the disruption of co-evolved interactions between them (Cruzan and Arnold 1999; Rand et al. 2004; Ellison and Burton 2006) as hypothesised by other intraspecific studies on Canary Island lizards (Pestano and Brown 1999; Gübitz et al. 2000). In this study, despite the deep mtDNA divergence, mtDNA lineage-specific microsatellite alleles were not found. Since greatest divergence is found between mtDNA lineages A and B, this implies that mtDNA lineage has no influence on spatial patterns of nuclear DNA in this species. Thus, the hypothesis of mito-nuclear incompatibility is rejected in this species.

Restriction of gene flow can be expected at the contact zone between species (e.g., Arntzen and Wallis 1999; Petit and Excoffier 2009). In this study, low rates of mtDNA gene flow suggested that gene flow between sites across the centre of the transect might be

restricted or absent. However, nuclear gene flow, which was observed at constant rates among sites and across the mtDNA contact zone, did not appear to be restricted. Moreover, a lack of significant mophological differentiation is not suggestive of a barrier to gene flow. Thus, the results indicated no obvious evidence of reproductive or other gene flow barriers.

Concordant mtDNA and nuclear gene trees can indicate substantial reproductive isolation and can be found among populations/subspecies (e.g., Smith et al. 2002; Glor et al. 2004; Yang and Rannala 2010). If the observed mtDNA break reflects speciation between members of each lineage, then the nuclear genome and/or morphology should also show concordant spatial patterns at the mtDNA contact zone. In studies of contact zones among different reptile taxa, it is typical to find concordant spatial patterns between nuclear and mtDNA (e.g., Leavitt et al. 2007; Joger et al. 2007). Given there was no evidence of genetic incompatibility or a gene flow barrier with respect to the nuclear genomes, the likelihood that this represents a case of incipient speciation is ruled out.

6.2 Possible causes of spatial discordance between mtDNA and nuclear DNA markers

The lack of correspondence between nuclear DNA and mtDNA at the contact zone could indicate different evolutionary histories for nuclear and mtDNA genomes (Roca et al. 2005; Di Candia and Routman 2007; Egger et al. 2007). There have been reports of geographical range shifts in nuclear DNA and/or morphology contact zones as a result of asymmetric gene flow (introgression) between differentiated populations leading to nuclear transition zones that are several kilometres away from the mtDNA contact zones (e.g., García-París et al. 2003; Kawakami et al. 2007; Ruegg 2007; Ganem et al. 2008). In this study, despite the inference of asymmetric gene flow from the mtDNA, a similar finding is not supported by microsatellites or morphology. Neither of these show evidence of

differentiaton between populations from north and south of the transect. Moreover, there is no evidence of a microsatellite transition zone outside the SE transect (see Appendix 4).

In other cases, spatial discordance between nuclear and mtDNA markers has been explained as a result of sex-biased dispersal (e.g., Lemaire et al. 2005; Gligor et al. 2009; Carvajal-Vallejos et al. 2010). This also includes cases of the homogenizing effect of male-biased dispersal leading to less structured distribution of nuclear DNA, such as in the bat *Myotis myotis* (Castella et al. 2001) and the mexican black iguana (*Ctenosaura pectinata*; Zarza et al. 2011). Nevertheless, no statistical support for sex-biased dispersal in *T. boettgeri* was obtained.

Thorpe et al. (2008; 2010) proposed the influence of ecological selection on nuclear DNA, which does not relate to allopatric divergence or mtDNA phylogeography, based on the studies of Martinique anole *Anolis roquest*. If this is the case here, spatial pattern of nuclear divergence should be concordant with the pattern of ecological variation across Gran Canaria. Microsatellites from individuals from high altitude (Tb01) and the north of Gran Canaria (SD01, JI01-JI03), (ecologically very different areas compared with the SE transect), are not differentiated from southern individuals. Therefore, it does not seem that nuclear variation in *T. boettgeri* has been influenced by the ecology of the island. However, this information is based on very few individuals/populations and so, further analyses are required before a more definitive conclusion can be obtained.

6.3 Factors maintaining the spatial pattern of mtDNA lineages

The persistence of spatial genetic variation results from a lack of gene flow between populations (Taberlet et al. 1995). Selection and dispersal were proposed as factors affecting rate of gene flow, which also influence structure, cline and maintenance of the contact zones (Haldane 1948; Barton and Hewitt 1982). In *T. boettgeri*, there is no

doubt that mtDNA contact zones have been maintained by low rates of mtDNA gene flow across the zones. It is clear that low gene flow is not a result of genetic incompatibility; thus, it does not support influence of endogenous selections. Moreover, lack of morphological differentiation between populations from different mtDNA lineages suggested that a mechanical barrier is unlikely.

Low dispersal is one of the factors proposed to maintain the contact zones between historically allopatric populations (Hare and Avise 1996; Sotka and Palumbi 2006). When dispersal is low, the introduced allele/haplotype may be lost, unless it benefits organisms in the new habitat (Holt and Gomulkiewicz 1997). In *T. boettgeri*, low levels of dispersal were detected using both mtDNA (female dispersal) and microsatellites, which in part help explain the long-term persistence of the mtDNA contact zone. Environmental homogeneity along the transect does not suggest that there is differential ecological adaptation of mtDNA lineages. Therefore, mtDNA gene flow should depend on rates of migration and dispersal. There is some (weak) evidence to suggest that females are less likely to disperse long-distances, thus mtDNA gene flow is reduced in relation to distances. Therefore, the contact zone may be trapped within the dispersal range preferred by females (5-10 km). Outside that range, number of female immigrants tended to be low. This will increase chance that the haplotype will be lost, particularly, if the majority of migrants were males.

Additional effects of low dispersal on maintaining of the contact zones was also reported. Based on interspecific studies, Bull (1991) proposed that a low dispersing species will be numerically disadvantaged and less able to penetrate the range of the resident species. This is because lower numbers of immigrants decrease the chance of (1) succesful competition for resources (including mating) with the resident population (Bull 1991; Bergstrom 1992), and (2) adaptation to the presence of other predation or parasitism beyond the edge of the contact zone (Paine 1974; Bergstrom 1992; Case, et al. 2005).

Where dispersal is low, weak intereactions with other species such as these will be sufficient to maintain the contact zones, where influence of environment and genetics are unclear (Bridle and Vines 2007). Thus, in *T. boettgeri*, it is suggested that low dispersal has contributed, at least in parts, to the long-term persistence of the mtDNA contact zone.

6.4 Conclusion

The detailed study of area of secondary contact between two mostly diverged mtDNA lineages of *T. boettgeri* revealed a strong mtDNA phylogeographical break with no transition in nuclear DNA background or morphology in correlation to spatial pattern of mtDNA. Thus, mtDNA lineages do not represent distinct species, but rather show population diversity within the species. This suggests that the mtDNA contact zone has not been maintained by incompatibility of nuclear genes or by mechanical barriers to breeding. The results also implied that *T. boettgeri* is a low dispersal species, thus, the mtDNA contact zone should be maintained by demographic factors rather than phenotypic or genotypic factors.

It is surprising that despite the long period of isolation that has given rise to mtDNA divergence there appears to be no similar pattern in the nuclear DNA. Nevertheless, it remains possible that microsatellites used in this study do not reflect the patterns across the entire nuclear genome. Based on studies of highly polymorphic markers in human (microsatellites, RFLP, SNPs), only a small percentage of genetic variation (10%) was due to differences between races, while the majority of the variation was accounted for within population diversity (Lewontin 1972; Nei and Roychoudhury 1974; Barbujani et al. 1997; Risch et al. 2002). Thus, fairly large numbers of markers are needed for detection of population structure in humans (at least 100 microsatellites, Bamshad et al. 2003). Clearly, mtDNA divergence among human populations is much lower than that observed here. Nevertheless, it is possible that by increasing the number of microsatellite

loci, markers may be found with similar genealogical histories that observed in the mtDNA.

References

Adams, S. M., Lindmeier, J. B., & Duvernell, D. D. (2006). Microsatellite analysis of the phylogeography, Pleistocene history and secondary contact hypotheses for the killifish, *Fundulus heteroclitus*. *Molecular Ecology,* 15: 1109–1123.

Albert, E. M., & Fernández, A. (2009). Evidence of cryptic speciation in a fossorial reptile: description of a new species of Blanus (Squamata: Amphisbaenia: Blanidae) from the Iberian Peninsula. *Zootaxa,* 2234: 56-68.

Albert, E. M., San Mauro, D., Garcia-Paris, M., Ruber, L., & Zardoya, R. (2009). Effect of taxon sampling on recovering the phylogeny of squamate reptiles based on complete mitochondrial genome and nuclear gene sequence data. *Gene,* 441 (1-2): 12-21.

Alexandrino, J., Baird, S. J., Lawson, L., Macey, J. R., Moritz, C., & Wake, D. B. (2005). Strong selection against hybrids at a hybrid zone in the *Ensatina* ring species complex and its evolutionary implications. *Evolution,* 39 (6): 1334-1347.

Altschul, S. F., Thomas, L. M., Alejandro, A. S., Jinghui, Z., Zhen, Z., Webb, M., David, J L. (1997). Grabbed BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Research,* 25: 3389-3402.

Anderson, C. L., Channing, A., & Zamuner, A. B. (2009). Life, death and fossilization on Gran Canaria – implications for Macaronesian biogeography and molecular dating. *Journal of Biogeography,* 36: 2189–2201.

Anguita, F., & Hernán, F. (2000). The Canary Islands origin: a unifying model. *Journal of Volcanology and Geothermal Research,* 103: 1 - 26.

Arias, C. F., Munoz, A. G., Jiggins, C. D., Mavarez, J., Bermingham, E., & Linares, M. (2008). A hybrid zone provides evidence for incipient ecological speciation in *Heliconius* butterflies. *Molecular Ecology,* 17: 4699–4712.

Arnaud, J. F., & Laval, G. (2004). Stability of genetic structure and effective population size inferred from temporal changes of microsatellite DNA polymorphisms in the land snail *Helix aspersa* (Gastropoda: Helicidae). *Biol. J. Linn. Soc,* 82: 89-102.

Arnold, M. L. (1994). Natural hybridization and Louisiana irises. *Bioscience,* 44: 141-147.

Arntzen, J. W., & Wallis, G. P. (1999). Geographic variation and taxonomy of crested newts (*Triturus cristatus* superspecies): morphological and mitochondrial DNA data. *Contributions to Zoology,* 68 (3): 181.

Avise, J. C. (1987). Identification and interpretation of mitochondrial DNA stocks in marine species. In H. Kumph, & E. L. Nakamura (Ed.), *Proceedings of the stock identification workshop* (pp. 105–136). Panama City: National Oceanographic and Atmospheric Administration.

Avise, J. C. (1989). Gree trees and organismal histories: A phylogenetic approach to population biology. *Evolution*, 43 (6): 1192-1208.

Avise, J. C., & Ball, R. M. (1990). Principles of genealogical concordance in species concepts and biolgical taxonomy. *Oxford Survey in Evolutionary Biology*, 7: 45-67.

Avise, J. C. (1992). Molecular Population Structure and the Biogeographic History of a Regional Fauna: A Case History with Lessons for Conservation Biology. *Oikos,* 63 (1): 62-76.

Avise, J. C., Walker, D., & Johns, G. C. (1998). Speciation durations and Pleistocene effects on vertebrate phylogeography. *Proc. R. Soc. Lond. B,* 265: 1707-1712.

Avise, J. C. (2001). *Phylogeography: The history and formation of species.* Cambridge, Massachusetts, USA: Harvard University Press.

Baack, E. J., & Rieseberg, L. H. (2007). A genomic view of introgression and hybrid speciation. *Current Opinion in Genetics & Development,* 17: 513-518.

Babik, W., Szymura, J. M., & Rafiński, J. (2003). Nuclear markers, mitochondrial DNA and male secondary sexual traits variation in a newt hybrid zone (*Triturus vulgaris* x *T. montandoni*). *Molecular Ecology,* 12: 1913-1930.

Babik, W., Branicki, W., Crnobrnja-Isailovic, J., Cogalniceanu, D., Sas, I., Olgun, K., Poyarkov, N. A., Garcia-Paris, M., Arntzen, J. W. (2005). Phylogeography of two European newt species — discordance between mtDNA and morphology. *Molecular Ecology,* 14: 2475–2491.

Báez, M., & Biscoito, M. (1993). First record of *Tarentola mauritanica* (Linneus, 1758) from the island of Madeira. *Macarones Congr., 1993,* summaries.

Báez, M., & Brown, R. P. (1997). Testing multivariate patterns of within-island differentiation in *Podarcis dugesii* from Madeira. *Journal of Evolutionary Biology,* 10 (4): 575–587.

Baha el Din, S. M. (1997) A new species of *Tarentola* (Squamata: Gekkonidae) from the western Desert of Egypt. *Afr. J. Herpetol.,* 46: 30-35.

Baker, R. J., & Davis, S. K. (1989). Ribosomal-DNA, mitochondrial-DNA, chromosomal, and allozymic studies on a contact zone in the pocket gopher, *Geomys. Evolution* , 43 (1): 63-75.

Bällard, J. W., Chernoff, B., & James, A. C. (2002). Divergence of mitochondrial DNA is not corroborated by nuclear DNA, morphology, or behavior in *Drosophila simulans. Evolution,* 56 (3): 527–545.

Ballard, J. W., & Whitlock, M. C. (2004). The incomplete natural history of mitochondria. *Molecular Ecology,* 13: 729-744.

Ballard, J. W. O., & Rand, D. M. (2005). The population biology of mitochondrial DNA and its phylogenetic implications. *Annual Review of Ecology, Evolution, and Systematics,* 36: 621-642.

Balogh, K., Ahijado, A., Casillas, R., & Fernández, C. (1999). Contribution to the chronology of the Basal Complex of Fuerteventura, Canary Islands. *Journal of Volcanology and Geothermal Research,* 90: 81-101.

Bamshad, M. J., Wooding, S., Watkins, W. S., Ostler, C. T., Batzer M. A., & Jorde, L. B. (2003). Human Population Genetic Structure and Inference of Group Membership. *Am. J. Hum. Genet.,* 72: 578–589.

Bandelt, H.-J., Forster, P., & Röhl, A. (1999). Median-Joining Networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.,* 16 (1): 37-48.

Barbanera, F., Zuffi, M. A., Guerrini, M., Gentilli, A., Tofanelli, S., Fasola, M., & Dini, F. (2009). Molecular phylogeography of the asp viper *Vipera aspis* (Linnaeus, 1758) in Italy: Evidence for introgressive hybridization and mitochondrial DNA capture. *Molecular Phylogenetics and Evolution,* 52: 103–114.

Barbujani, G., Magagni A., Minch E., & Cavalli-Sforza, L. L. An apportionment of human DNA diversity. *Proc. Natl. Acad. Sci. USA,* 94: 4516–4519.

Barton, N H, & G M Hewitt. (1982). A measurement of dispersal in the grasshopper *Podisma pedestris* (Orthoptera: Acrididae). *Heredity*, 48 (2): 237-249.

Barton, N., & Jones, J. S. (1983). Mitochondrial DNA: new clues about evolution. *Nature,* 306: 317-318.

Barton, N. H., & Hewitt, G. M. (1985). Analysis of Hybrid Zones. *Annual Review of Ecology and Systematics,* 16: 133-48.

Barton, N. H., & Hewitt, G. M. (1989). Adaptation, speciation and hybrid zones. *Nature,* 341: 497.

Barton, N. H., & Gale, K. S. (1993). Genetic analysis of hybrid zones. In *Hybrid zones and the evolutionary process* (ed. R. G. Harrison), 13-45. Oxford University Press.

Barton, N. H. (2000). Estimating multilocus linkage disequilibria. *Heredity,* 84: 373-389.

Barton, N. H. (2001). The role of hybridization in evolution. *Molecular Ecology,* 10: 551-568.

Bates, J. M., Haffer, J., & Grismer, E. (2004). Avian mitochondrial DNA sequence divergence across a headwater stream of the Rio Tapajós, a major Amazonian river. *J Ornithol,* 145: 199-205.

Bauer, A. M., de Silva, A., Greenbaum, E., & Jackman, T. (2007). A new species of day gecko from high elevation in Sri Lanka, with a preliminary phylogeny of Sri Lankan *Cnemaspis* (Reptilia, Squamata, Gekkonidae). *Mitt. Mus. Nat.kd. Berl., Zool. Reihe,* 83: 22-32.

Beerli, P., & Felsenstein, J. (1999). Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. *Genetics,* 152 (2): 763-773.

Beerli, P., & Felsenstein, J. (2001). Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. *Proc. Natl. Acad. Sci. USA,* 98 (8): 4563-4568.

Beerli, P. (2008). Migrate version 3.0: a maximum likelihood and Bayesian estimator of gene flow using the coalescent. Distributed over the internet at http://popgen.scs.edu/migrate.html.

Bennett, P. (2000). Demystified: Microsatellites. *J Clin Pathol.,* 53: 177-183.

Bensasson, D., Zhang, D.-X., Hartl, D. L., & Hewitt, G. M. (2001). Mitochondrial pseudogenes: evolution's misplaced witnesses. *TRENDS in Ecology & Evolution,* 16 (6): 314-321.

Bensch, S., Andersson, T., & Akesson, S. (1999). Morphological and molecular variation across a migratory divide in willow warblers, Phylloscopus trochilus. *Evolution,* 53 (6): 1925-1935.

Bergstrom, B. J. (1992). Parapatry and encounter competition between chipmunk (*Tamias*) species and the hypothesized role of parasitism. *Am. Midl. Nat.* 128: 168–179.

Bernardi, G., Sordino, P., & Powers, D. A. (1993). Concordant mitochondrial and nuclear DNA phylogenies for populations of the teleost fish *Fundulus heteroclitus. Proc. Natl. Acad. Sci. USA,* 90: 9271-9274.

Berry, E. A., Guergova-Kuras, M., Huang, L.-s., & Crofts, A. R. (2000). Structure and function of cytochrome bc complexes. *Annu. Rev. Biochem.,* 69: 1005–1035.

Berthier, P., Excoffier, L., & Ruedi, M. (2006). Recurrent replacement of mtDNA and cryptic hybridization between two sibling bat species *Myotis myotis* and *Myotis blythii. Proceeding of The Royal Society B,* 273: 3101-3109.

Bittner, T. D., & King, R. B. (2003). Gene flow and melanism in garter snakes revisited: a comparison of molecular markers and island vs. coalescent models. *Biological Journal of the Linnean Society,* 79: 389–399.

Bloor, P., & Brown, R. P. (2005). Morphological valriation in *Gallotia atlantica* from the volcanic island of Lanzarote: subspecies designations and recent lava flows. *Biological Journal of the Linnean Society,* 85: 395-406.

Bloor, P., De Laguna, I. H.-B., & Kemp, S. J. (2006). Highly polymorphic tetranucleotide microsatellite loci for the eastern Canary Island lizard, *Gallotia atlantica. Molecular Ecology,* 6: 737-739.

Bloor, P., & Dávila, A. (2008). Polymorphic microsatellite markers for the lizard *Psammodromus algirus* (Squamata: Lacertidae). *Molecular Ecology Resources,* 8: 631-633.

Bloor, P., Kemp, J., & Brown, R. P. (2008). Recent volcanism and mitochondrial DNA structuring in the lizard *Gallotia atlantica* from the island of Lanzarote. *Molecular Ecology,* 17: 854-866.

Borge, T., Lindroos, K., Nadvornik, P., Syvanen, A. C., & Saetre, G. P. (2005). Amount of introgression in flycatcher hybrid zones reflects regional differences in pre and post–zygotic barriers to gene exchange. *Journal of Evolutionary Biology,* 18: 1416 –1424.

Bradley, R. D., & Baker, R. J. (2001). A test of the genetic species concept: cytochrome b sequences and mammals. *J. Mammal*, 82: 960–973.

Branch, L. C., Clark, A. M., Moler, P. E., & Bowen, B. W. (2003). Fragmented landscapes, habitat specificity, and conservation genetics of three lizards in Florida scrub. *Conservation Genetics,* 4: 199–212.

Bridle, J. R., Garn, A.-K., Monk, K. A., & Butlin, R. K. (2001). Speciation in *Chitaura* grasshoppers (Acrididae: Oxyinae) on the island of Sulawesi: colour patterns, morphology and contact zone. *Biological Journal of the Linnean Society,* 72: 373-390.

Bridle, J. R., & Vines, T. H. (2007). Limits to evolution at range margins: when and why does adaptation fail?. *Trends in Ecology & Evolution,* 22 (3): 140-147.

Brochu, C. A., & McEachran, J. D. (2000). Phylogenetic relationships and divergence timing of *Crocodylus* based on morphology and the fossil record. *Copeia,* 2000 (3): 657-673.

Brower, A. V. (1994). Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proc. Natl. Acad. Sci. USA,* 91: 6491-6495.

Brower, A. V. (1996). Parallel race formation and the evolution of mimicry in *Heliconius* butterflies: A phylogenetic hypothesis from mitochondrial DNA sequences. *Evolution,* 50 (1): 195-221.

Brown, R. P., & Thorpe, R. S. (1991). Within-island microgeographic variation in the colour pattern of the skink, *Chalcides sexlineatus*: Pattern and cause. *Journal of Evolutionary Biology,* 4: 557-574.

Brown, R. P., Thorpe, R. S., & Báez, M. (1991). Parallel within-island microevolution of lizards on neighbouring islands. *Nature*, 352: 60-62.

Brown, R. P., Thorpe, R. S., & Báez, M. (1993) Patterns and causes of morphological population differentiation in the Tenerife skink, *Chalcides viridanus*. *Biol. J. Linn. Soc.,* 50: 313-328.

Brown, R. P. (1996). Thermal biology of the gecko *Tarentola boettgeri*: comparisons among populations from different elevations within Gran Canaria. *Herpetologica,* 52 (3): 396-405.

Brown, R. P., & Pestano, J. (1998). Phylogeography of skinks (*Chalcides*) in the Canary Islands inferred from mitochondrial DNA sequences. *Molecular Ecology*, 7: 1183–1191.

Brown, R. P., Campos-Delagado, R., & Pestano, J. (2000). Mitochondrial DNA evolution and population history of the Tenerife skink *Chalcides viridanus*. *Molecular Ecology,* 9: 1061-1067.

Brown, R. P., Saurez, N. M., & Pestano, J. (2002). The Atlas mountains as a biogeographical divide in North-West Africa: evidence from mtDNA evolution in the Agamid lizard *Agama impalearis*. *Molecular Phylogenetics and Evolution,* 24: 324-332.

Brown, R. P., Hoskisson, P. A., Welton, J.-H., & Báez, M. (2006). Geological history and within-island diversity: a debris avalanche and the Tenerife lizard *Gallotia galloti*. *Molecular Ecology,* 15: 3631-3640.

Bull, C M. (1991). Ecology of Parapatric Distributions. *Annual Review of Ecology and Systematics,* 22: 19-36.

Caparroz, R., Miyaki, C. Y., & Baker, A. J. (2009). Contrasting phylogeographic patterns in mitochondrail DNA and microsatellites: evidence of female philopatry and male-biased gene flow among regional populations of the blue-and-yellow macaw (Psittaciformes: *Ara ararauna*) in Brazil. *The Auk,* 126 (2): 359-370.

Carlsson, M., Söderberg, L., & Tegelström, H. (2004). The genetic structure of adders (*Vipera berus*) in Fennoscandia: congruence between different kinds of genetic markers. *Molecular Ecology,* 13: 3147–3152.

Carranza, S., Arnold, E. N., Mateo, J. A., & Lòpez-Jurado, L. F. (2000). Long-distance colonization and radiation in gekkonid lizards, *Tarentola* (Reptilia: Gekkonidae), revealed by mitochondrial DNA sequences. *Proceedings of the Royal Society of London Series B-Biological Sciences* , 267: 637-649.

Carranza, S., Arnold, E. N., Mateo, J. A., & Geniez, P. (2002). Relationships and evolution of the North African geckos, *Geckonia* and *Tarentola* (Reptilia: Gekkonidae), based on mitochondrial and nuclear DNA sequences. *Molecular Phylogenetics and Evolution,* 23: 244-256.

Carranza, S., & Arnold, E. N. (2006). Systematics, biogeography, and evolution of *Hemidactylus* geckos (Reptilia: Gekkonidae) elucidated using mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution,* 38: 531-545.

Carrascal, L. M., Seoane, J., Palomino, D., & Polo, V. (2008). Explanations for bird species range size: ecological correlates and phylogenetic effects in the Canary Islands. *Journal of Biogeography,* 35: 2061–2073.

Čarson, H. L., Lockwood, J. P., & Carddock, E. M. (1990). Extinction and recolonization of local populations on a growing shield volcano. *Proc. Nati. Acad. Sci. USA,* 87: 7055-7057.

Carvajal-Vallejos, F. M., Duponchelle, F., Torrico Ballivian, J. P., Hubert, N., Rodríguez, J. N., Berrebi, P., et al. (2010). Population genetic structure of *Cichla pleiozona* (Perciformes: Cichlidae) in the Upper Madera basin (Bolivian Amazon): Sex-biased dispersal? *Molecular Phylogenetics and Evolution,* 57: 1334–1340.

Case, T. J., Holt, R. D., McPeek, M. A., & Keitt, T. H. (2005). The community context of species' borders: ecological and evolutionary perspectives. *OIKOS* 108: 28-46.

Castella, V., Ruedi, M., & Excoffier, L. (2001). Contrasted pattern of mitochondrial and nuclear structure among nursery colonies of the bat *Myotis myotis*. *Journal of Evolutionary Biology,* 14: 708–720.

Castresana, J. (2001). Cytochrome b phylogeny and the taxonomy of great apes and mammals. *Mol. Biol. Evol.,* 18 (4): 465–471.

Cejudo, D., & Márquez, R. (2001). Sprint performance in the lizards *Gallotia simonyi* and *Gallotia stehlini* (*Lacertidae*): Implications for species management. *Herpetologica,* 57 (1): 87-98.

Chow, S., & Takeyama, H. (2000). Nuclear and mitochondrial DNA analyses reveal four genetically separated breeding units of swordfish. *Journal of Fish Biology,* 56: 1087-1098.

Cicero, C. (2004). Barriers to sympatry between avian sibling species (Paridae: Baeolophus) in local secondary contact. *Evolution,* 58 (7): 1573–1587.

Ciofi, C., & Bruford, M. W. (1999). Genetic structure and gene flow among Komodo dragon populations inferred by microsatellite loci analysis. *Molecular Ecology,* 8: S17–S30.

Clement, M., Posada, D., & Crandall, K. A. (2000). TCS: a computer program to estimate gene genealogies. *Molecular Ecology,* 9: 1657–1659.

Cockerham, C. C. (1969). Variance of gene frequencies. *Evolution,* 23: 72-83.

Cockerham, C. C. (1973). Analysis of gene frequencies. *Genetics,* 74: 679-700.

Collura, R. V., & Stewart, C.-B. (1995). Insertions and duplications of mtDNA in the nuclear genomes of Old World monkeys and hominoids. *Nature,* 378: 485 - 489.

Collura, R. V., Auerbach, M. R., & Stewart, C.-B. (1996). A quick, direct method that can differentiate expressed mitochondrial genes from their nuclear pseudogenes. *Current Biology,* 6 (10): 1337–1339.

Comas, D., Plaza, S., Wells, R. S., Yuldaseva, N., Lao, O., Calafell, F., et al. (2004). Admixture, migrations, and dispersals in Central Asia: evidence from maternal DNA lineages. *European Journal of Human Genetics,* 12: 495-504.

Contreras-Díaz, H. G., Moya, O., Oromí, P. & Juan, C. (2003). Phylogeography of the endangered darkling beetle species of *Pimelia* endemic to Gran Canaria (Canary Islands). *Molecular Ecology,* 12: 2131–2143.

Cox, S. C., Carranza, S., & Brown, R. P. (2010). Divergence times and colonization of the Canary Islands by *Gallotia* lizards. *Molecular Phylogenetics and Evolution,* 56: 747–757.

Coyne, J. A. (1993). The genetics of an isolating mechanism between two sibling species of *Drosophila*. *Evolution,* 47: 778–788.

Cronin, M. A. (1992). Intraspecific Variation in Mitochondrial DNA of North American Cervids. *Journal of Mammalogy,* 73 (1): 70-82.

Cruzan, M. B., & Arnold, M. L. (1999). Consequences of cytonuclear epistasis and assortative mating for the genetic structure of hybrid populations. *Heredity,* 82: 36–45.

Daguin, C., Bonhomme, F., & Borsa, P. (2001). The zone of sympatry and hybridization of *Mytilus edulis* and *M. galloprovincialis*, as described by intron length polymorphism at locus *mac-1*. *Heredity,* 86: 342-354.

Dasmahapatra, K. K., Blum, M. J., Aiello, A., Hackwell, S., Davies, N., Bermingham, E. P., & Mallet., J. (2002). Inferences from a rapidly moving hybrid zone. *Evolution,* 56 (4): 741-753.

Davison, A. (2000). An East-West distribution of divergent mitochondrial haplotypes in British populations of the land snail, *Cepaea nemoralis* (Pulmonata). *Biol. J. Linn. Soc.,* 70: 697– 706.

De Chaine, E. G., & Martin, A. P. (2006). Using coalescent simulations to test the impact of quaternary climate cycles on divergence in an Alpine plant-insect association. *Evolution,* 60 (5): 1004–1013.

De La Rúa, P., Galián, J., Serrano, J., & Moritz, R. F. (2001). Genetic structure and distinctness of *Apis mellifera* L. populations from the Canary Islands. *Molecular Ecology,* 10: 1733–1742.

De Queiroz, K. (2007). Species Concepts and Species Delimitation. *Syst. Biol.,* 56 (6): 879–886.

Demastes, J. W., Eastman, J. M., & East, J. S. (2007). Phylogeography of the Blue-spotted Salamander, *Ambystoma laterale* (Caudata: Ambystomatidae). *Am. Midl. Nat.,* 157: 149–161.

Demboski, J. R., & Cook, J. A. (2001). Phylogeography of the dusky shrew, *Sorex monticolus* (Insectivora, Soricidae): insight into deep and shallow history in northwestern North America. *Molecular Ecology,* 10: 1227–1240.

Dépraz, A., Hausser, J., & Pfenninger, M. (2009). A species delimitation approach in the *Trochulus sericeus/hispidus* complex reveals two cryptic species within a sharp contact zone. *BMC Evolutionary Biology,* 9 (171): doi:10.1186/1471-2148-9-171.

Dessauer, H. C., & Cole, C. J. (1991). Genetics of whiptail lizards (*Reptilia: Teiidae: Cnemidophorus*) in a hybrid zone in Southwestern New Mexico. *Copeia,* 1991 (3): 622-637.

DeWoody, J. A., Chesser, R. K., & Baker, R. J. (1999). A translocated mitochondrial cytochrome b pseudogene in voles (Rodentia: Microtus). *J Mol Evol,* 48: 380–382.

DeWoody, J., Nason, J. D., & Hipkins, V. D. (2006). Mitigating scoring errors in microsatellite data from wild populations. *Molecular Ecology Notes,* 6: 951–957.

Di Candia, M. R., & Routman, E. J. (2007). Cytonuclear discordance across a leopard frog contact zone. *Molecular Phylogenetics and Evolution,* 45: 564–575.

Dobson, F. S. (1982). Competition for mates and predominant juvenile male dispersal in mammals. *Anim. Behav.,* 30: 1183– 92.

Dobzhansky, T. (1936). Studies on hybrid sterility. II. Localization of sterility factors in *Drosophila pseudoobscura* hybrids. *Genetics,* 21: 113–135.

Dolman, G., & Phillips, B. (2004). Single copy nuclear DNA markers characterized for comparative phylogeography in Australian wet tropics rainforest skinks. *Molecular Ecology Notes,* 4 (2): 185-187.

Donnelly, P., & Tavaré, S. (1995). Coalescents and genealogical structure under neutrality. *Annu. Rev. Genet.,* 29: 401-421.

Doughty, P., Sinervo, B., & Burghardt, G. M. (1994). Sex-biased dispersal in a polygynous lizard, *Uta stansburiana. Animal Behaviour,* 47: 227-229.

Dowling, T. E., & Secor, C. L. (1997). The role of hybridization and introgression in the diversification of animals. *Annual Review of Ecology and Systematics,* 28: 593 - 619.

Dubey, S., & Shine, R. (2010). Restricted dispersal and genetic diversity in populations of an endangered montane lizard (*Eulamprus leuraensis, Scincidae*). *Molecular Ecology,* 19: 886-897.

Duméril, A.M.C & Bibron, G. (1836). Erpétologie générale our histoire naturelle complète des reptiles. Librairie Encyclopédique de Roret, Paris.

Durand, J. D., Collet, A., Chow, S., Guinand, B., & Borsa, P. (2005). Nuclear and mitochondrial DNA markers indicate unidirectional gene flow of Indo-Pacific to Atlantic bigeye tuna (*Thunnus obesus*) populations, and their admixture off south Africa. *Marine Biology,* 147: 313-322.

Edwards, S. V., Arctander, P., & Wilson, A. C. (1991). Mitochondrial resolution of a deep branch in the genealogical tree for perching birds. *Proceedings of the Royal Society of London Series B,* 243: 99–107.

Edwards, S. V., & Beerli, P. (2000). Perspective: gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. *Evolution,* 54 (6): 1839-1854.

Egger, B., Koblmuller, S., Sturmbauer, C., & Sefc, K. (2007). Nuclear and mitochondrial data reveal different evolutionary processes in the Lake Tanganyika cichlid genus *Tropheus. BMC Evol. Biol.,* 7: 137.

Ellison, C. K., & Burton, R. S. (2006). Disruption of mitochondrial function in interpopulation hybrids of *Tigriopus californicus. Evolution,* 60: 1382–1391.

Emerson, B. C., Oromi, P., & Hewitt, G. M. (1999). MtDNA Phylogeography and recent intra-island diversification among Canary Island *Calathus* beetles. *Molecular Phylogenetics and Evolution,* 13 (1): 149–158.

Emerson, B. C., Oromí, P. & Hewitt, G. M. (2000). Tracking colonization and diversification of insect lineages on islands: mitochondrial DNA phylogeography of *Tarphius canariensis* (Coleoptera: Colydiidae) on the Canary Islands. *Proc R. Soc. Lond. B,* 267: 2199-2205.

Emerson, B. C., & Oromí, P. (2005). Diversification of the forest beetle genus *Tarphius* on the Canary Islands, and the evolutionary origins of island endemics. *Evolution,* 59 (3): 586–598.

Emerson, B. C., Forgie, S., Goodacre, S., & Oromí, P. (2006). Testing phylogepgraphic predictions on an active volcanic island: *Brachyderes rugatus* (Coleoptera: Curculionidae) on La Palma (Canary Islands). *Molecular Ecology,* 15: 449-458.

Endler, J. A. (1977). *Geographic variation, speciation and clines.* Princeton, NJ., Princeton University Press.

Endler, J. A. (1982). Problems in distinguishing historical from ecological factors in biogeography. *American Zoologist,* 22 (2): 441-452.

Esposti, M. D., Vries, S. D., Crimi, M., Ghelli, A., Patarnello, T., & Meyer, A. (1993). Mitochondrial cytochrome b: evolution and structure of the protein. *Biochimica et Biophysica Acta,* 1143: 243-271.

Estoup, A., Jarne, P., & Cornuet, J.-M. (2002). Homoplasy and mutation model at microsatellite loci and their consequences for population genetics analysis. *Molecular Ecology,* 11: 1591–1604.

Estoup, A., Tailliez, C., Cornuet, J.-M., & Solignac, M. (1995). Size homoplasy and mutational processes of interrupted microsatellites in two bee species, *Apis mellifera* and *Bombus terrestris* (*Apidae*). *Molecular Biology and Evolution,* 12 (6): 1074 –1084.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology,* 14: 2611–2620.

Excoffier, L. (2001). Analysis of population subdivision. In D. J. Balding, M. Bishop, & C. Cannings, *Handbook of statistical genetics* (Vols. 271-307). Chichester, UK: John Wiley & Sons.

Excoffier, L., & Lischer, H. E. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources,* 10: 564-567.

Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics,* 131: 479-491.

Favre, L., Balloux, F., Goudet, J., & Perrin, N. (1997). Female-biased dispersal in the monogamous mammal *Crosidura russula*: evidence from field data and microsatellite patterns. *Proc. R. Soc. Lond. B,* 264: 127–32.

Fernandopulle, D. (1976). Climate characteristics of the Canary Islands. In G. Kunkel, *Biogeography and Ecology in the Canary Islands* (pp. 185-206). Junk: The Hague.

Filardi, C. E., & Smith, C. E. (2005). Molecular phylogenetics of monarch *Xycatchers* (genus *Monarcha*) with emphasis on Solomon Island endemics. *Molecular Phylogenetics and Evolution,* 37: 776–788.

Fitzpatrick, B. M., Placyk, J. S., Niemiller, M. L., Casper, G. S., & Burghardt, G. M. (2008). Distinctiveness in the face of gene flow: hybridization between specialist and generalist gartersnakes. *Molecular Ecology,* 17: 4107–4117.

Fleischer, R. C., Rothstein, S. I., & Miller, L. S. (1991). Mitochondrial DNA variation indicates gene flow across a zone of known secondary contact between two subspecies of the brown-headed cowbird. *The Condor,* 93 (1): 185-189.

Francisco-Ortega, J., Santos-Guerra, A., Kim, S.-C., & Crawford, D. J. (2000). Plant genetic diversity in the Canary Islands: A conservation perspective. *American Journal of Botany,* 87 (7): 909–919.

Fritts, T. H. (1984). Evolutionary divergence of giant tortoises in Galapagos. *Biological Journal of the Linnean Society,* 21 (1-2): 165–176.

Fritz, U., Ayaz, D., Buschbom, J., Kami, H. G., Mazanaeva, L. F., Aloufi, A. A., et al. (2008). Go east: phylogeographies of *Mauremys caspica* and *M. rivulata* – discordance of morphology, mitochondrial and nuclear genomic markers and rare hybridization. *J. Evol. Biol.,* 21: 527–540.

Fu, Y. X., & Li, W. H. (1999). Coalescing into the 21st century: an overview and prospects of coalescent theory. *Theor. Popul. Biol.,* 56(1): 1-10.

Furman, A., Postawa, T., Öztunc, T., & Çoraman, E. (2010). Cryptic diversity of the bent-wing bat, *Miniopterus schreibersii* (Chiroptera: Vespertilionidae), in Asia Minor. *BMC Evolutionary Biology,* 10: 121.

Ganem, G., Litel, C., & Lenormand, T. (2008). Variation in mate preference across a house mouse hybrid zone. *Heredity,* 100(6): 594-601.

García-París, M., Good, D. A., Parra-Olea, G., & Wake, D. B. (2000). Biodiversity of Costa Rican salamanders: implications of high levels of genetic differentiation and phylogeographical structure for species formation. *PNAS,* 97 (4): 1640-1647.

García-París, M., Alcobendas, M., Buckley, D., & Wake, D. B. (2003). Dispersal of viviparity across contact zones in Iberian populations of fire salamanders (*Salamandra*) inferred from discordance of genetic and morphological traits. *Evolution,* 57 (1): 129–143.

Garcia Rodriguez, J.-L., Hernandez, J. H., Cabrera Armas, L. G., Diaz de la Paz, A. & Perez, L. A. (1990) *Atlas interinsular de Canarias.* Editorial Interinsular Canarias, S.A., Santa Cruz de Tenerife.

Gardner, M. G., Cooper, S. J., Bull, C. M., & Grant, W. N. (1999). Isolation of microsatellite loci from a social lizard, *Egernia stokesii,* using a modified enrichment pricedure. *The Journal of Heredity,* 90 (2): 301-304.

Gay, L., Neubauer, G., Zagalska-Neubauer, M., Debain, C., Pons, J. M., David, P., Crochet, P. A. (2007). Molecular and morphological patterns of introgression between two large white-headed gull species in a zone of recent secondary contact. *Molecular Ecology,* 16: 3215–3227.

Gershoni, M., Templeton, A. R., & Mishmar, D. (2009). Mitochondrial bioenergetics as a major motive force of speciation. *BioEssays,* 31: 642–650.

Gibbs, H. L., Corey, S. J., Blouin-Demers, G., Prior, K. A., & Weatherhead, P. J. (2006). Hybridization between mtDNA-defined phylogeographic lineages of black ratsnakes (*Pantherophis sp.*). *Molecular Ecology,* 15: 3755–3767.

Gligor, M., Ganzhorn, J. U., Rakotondravony, D., Ramilijaona, O. R., Razafimahatratra, E., Zischler, H., & Hapke, A. (2009). Hybridization between mouse lemurs in an ecological transition zone in southern Madagascar. *Molecular Ecology,* 18: 520–533.

Glor, R. E., Gifford, M. E., Larson, A., Losos, J. B., Schettino, L. R., Lara, A. R., Jackman, T R. (2004). Partial island submergence and speciation in an adaptive radiation: a multilocus analysis of the Cuban green anoles. *Proc. R. Soc. Lond. B,* 271: 2257-2265.

Godinho, R., Mendonca, B., Crespo, E. G., & Ferrand, N. (2006). Genealogy of the nuclear b-fibrinogen locus in a highly structured lizard species: comparison with mtDNA and evidence for intragenic recombination in the hybrid zone. *Heredity*, 96 (6): 454-463.

Godinho, R., Crespo, E. G., & Ferrand, N. (2008). The limits of mtDNA phylogeography: complex patterns of population history in a highly structured Iberian lizard are only revealed by the use of nuclear markers. *Molecular Ecology,* 17: 4670–4683.

Goldstein, D. B., & Schlötterer, C. (2001). *Microsatellites: Evolution and Applications* (3 ed.). Oxford: Oxford University Press.

Gonzalez, E. G., & Zardoya, R. (2007). Relative role of life-histiry traits and historical factors in shaping genetic population structure of sardine (*Sardina pilchardus*). *BMC Evolutionary Biology,* 7 (197): doi:10.1186/1471-2148-7-197.

Gonzalez, P., Pinto, F., Nogales, M., Jimenez-Asensio, J., Hernandez, M., & Cabrera, V. M. (1996). Phylogenetic Relationships of the Canary Islands Endemic Lizard Genus *Gallotia* (Sauria: Lacertidae), Inferred from Mitochondrial DNA Sequences. *Molecular Phylogenetics and Evolution,* 6 (1): 63–71.

Good, J. M., & Sullivan, J. (2001). Phylogeography of the red-tailed chipmunk (*Tamias ruficaudus*), a northern Rocky Mountain endemic. *Molecular Ecology,* 10: 2683–2695.

Good, J. M., Hird, S., Reid, N., Demboski, J. R., Steppan, S. J., Martin-Nims, T. R., Sullivan, J. (2008). Ancient hybridization and mitochondrial capture between two species of chipmunks. *Molecular Ecology* , 17: 1313-1327.

Goudet, J. (1995). Fstat (version 1.2): a computer program to calculate F-statistics. *Journal of Heredity,* 86 (6): 485-486.

Goudet, J., Perrin, N., & Waser, P. (2002). Tests for sex-biased dispersal using bi-parentally inherited genetic markers. *Molecular Ecology,* 11: 1103–1114.

Grahame, J. W., Wilding, C. S., & Butlin, R. K. (2006). Adaptation to a steep environmental gradient and an associated barrier to gene exchange in *Littorina saxatilis*. *Evolution,* 60 (2): 268–278.

Greenwood, P. J. (1980). Mating systems, philopatry and dispersal in birds and mammals. *Anim. Behav.*, 28: 1140–62.

Griebeler, E. M., Müller, J. C., & Seitz, A. (2006). Spatial genetic patterns generated by two admixing genetic lineages: a simulation study. *Conservation Genetics,* 7 (5): 753-766.

Grossman, L., Wildman, D. E., Schmidt, T. R., & Goodman, M. (2004). Accelerated evolution of the electron transport chain in anthropoid primates. *TRENDS in Genetics,* 20 (11): 578-585.

Gübitz, T. (1999, September). Molecular and Morphological Evolution in *Tarentola boettgeri* and *T. delalandii* in the Canary Archipelago. *PhD Thesis.* Bangor, United Kingdom: University of Wales.

Gübitz, T., Thorpe, R. S., & Malhotra, A. (2000). Phylogeography and natural selection in the Tenerife gecko *Tarentola delalandii*: testing historical and adaptive hypotheses. *Molecular Ecology,* 9: 1213–1221.

Gübitz, T., Thorpe, R. S., & Malhotra, A. (2005). The dynamics of genetic and morphological variation on volcanic islands. *Proceedings of The Royal Society B,* 272: 751–757.

Guillaume, C. P., Heulin, ,. B., Arrayago, M. J., Bea, A., & Brana, F. (2000). Refuge areas and suture zones in the Pyrenean and Cantabrian regions: geographic variation of the female MPI sex-linked alleles among oviparous populations of the lizard *Lacerta* (Zootoca) vivipara. *Ecography,* 23: 3–10.

Guillou, H., Carracedo, J. C., Torrado, F. P., & Badiola, E. R. (1996). K-Ar ages and magnetic stratigraphy of a hotspot-induced, fast grown oceanic island: El Hierro, Canary Islands. *Journal of Volcanology and Geothermal Research,* 73: 141-155.

Gum, B., Gross, R., & Kuehn, R. (2005). Mitochondrial and nuclear DNA phylogeography of European grayling (*Thymallus thymallus*): evidence for secondary contact zones in Europe. *Molecular Ecology,* 14: 1707-1725.

Guo, S. W., & Thompson, E. A. (1992). Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics,* 48 (2): 361-72.

Gutiérrez-Rodríguez, C., & Lasker, H. R. (2004). Microsatellite variation reveals high levels of genetic variability and population structure in the gorgonian coral *Pseudopterogorgia elisabethae* across the Bahamas. *Molecular Ecology,* 13: 2211–2221.

Gutiérrez-Rodríguez, C., Shearer, A. E., Morris, M. R., & De Queiroz, K. (2008). Phylogeography and monophyly of the swordtail fish species *Xiphophorus birchmanni* (Cyprinodontiformes, Poeciliidae). *Zoologica Scripta,* 37 (2): 129–139.

Haldane, J. B. S. (1948). The theory of a cline. *J. Genet.*, 48: 277-284.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser.,* 41: 95-98.

Handley, L. J., & Perrin, N. (2007). Advances in our understanding of mammalian sex-biased dispersal. *Molecular Ecology,* 16: 1559–1578.

Hare, M. P., & Avise, J. C. (1996). Molecular genetic analysis f a stepped multilocus cline in the American oyster (*Crassostre virginica*). *Evolution,* 50 (6): 2305-2315.

Hasbún, C. R., Gómez, A., Köhler, G., & Lunt, D. H. (2005). Mitochondrial DNA phylogeography of the Mesoamerican spiny-tailed lizards (*Ctenosaura quinquecarinata* complex): historical biogeography, species status and conservation. *Molecular Ecology,* 14: 3095–3107.

Hastings, W. (1970). Monte Carlo sampling methods using Markov chains and their applications. *Biometrika,* 57: 97–109.

Heaney, L. R., & Timm, R. M. (1985). Morphology, genetics, and ecology of pocket gophers (genus *Geomys*)in a narrow hybrid zone. *Biological Journal of the Linnean Sociely,* 25: 301-317.

Helbig, A. J., Salomon, M., Bensch, S., & Seibold, I. (2001). Male-biased gene flow across an avian hybrid zone: evidence from mitochondrial and microsatellite DNA. *J. Evol. Biol.,* 14: 277-287.

Hewitt, G. M. (1996). Some genetic consequences of ice ages, and their tole in divergence and speciation. *Biological Journal of the Linnean Society,* 58: 247-276.

Hewitt, G. M. (2000). The genetic legacy of the Quaternary ice ages. *Nature,* 405: 907–913.

Hewitt, G. M. (2001). Speciation, hybrid zones and phylogeography - or seeing gene in space and time. *Molecular Ecology,* 10: 537-549.

Hey, J. (2006). Recent advances in assessing gene flow between diverging populations and species. *Current Opinion in Genetics and Development,* 16: 592–596.

Hird, S., & Sullivan, J. (2009). Assessment of gene flow across a hybrid zone in red-tailed chipmunks (*Tamias ruficaudus*). *Molecular Ecology,* 18: 3097–3109.

Hoehn, M., Sarre, S. D., & Henle, K. (2007). The tales of two geckos: does dispersal prevent extinction in recently fragmented populations? *Molecular Ecology,* 16 (16): 3299–3312.

Hoernle, K., & Schmincke, H.-U. (1993a). The role of partial melting in the 15-Ma geochemial evolution of Gran Canaria: a blob model for the Canary hotspot. *Journal of Petrology,* 34: 599-626.

Holt, R. D., & Gomulkiewicz R. (1997). How Does Immigration Influence Local Adaptation? A Reexamination of a Familiar Paradigm. *The American Naturalist,* 149 (3): 563-572.

Howell, N. (1989). Evolutionary conservation of protein regions in the proton-motive cytochrome b and their possible roles in redox catalysis. *J. Mol. Evol.,* 29: 157-169.

Howes, B. J., Lindsay, B., & Lougheed, S. C. (2006). Range-wide phylogeography of a temperate lizard, the Wve-lined skink (*Eumeces fasciatus*). *Molecular Phylogenetics and Evolution,* 40: 183–194.

Hudson, R. R. (1990). Gene Genealogies and the coalescent process. *Oxf. Surv. Evol. Biol.,* 7: 1-44.

Huelsenbeck, J. P., Ronquist, F., Nielsen, R., & Bollback, J. P. (2001). Bayesian inference of phylogeny and its impact on evolutionary biology. *Science,* 294: 2310-2314.

Irwin, D. M., Kocher, T. D., & Wilson, A. C. (1991). Evolution of the cytochrome b gene of mammals. *J Mol Evol,* 32: 128-144.

James, C. H., & Moritz, C. (2000). Intraspecific phylogeography in the sedge frog *Litoria fallax* (*Hylidae*) indicates pre-Pleistocene vicariance of an open forest species from eastern Australia. *Molecular Ecology,* 9 (3): 349–358.

Janzen, F. J., Krenz, J. G., Haselkorn, T. S., Brodie, J. E., & Brodie, I. E. (2002). Molecular phylogeography of common garter snakes (*Thamnophis sirtalis*) in western North America: implications for regional historical forces. *Molecular Ecology,* 11: 1739–1751.

Jiggins, C. D., McMillan, W. O., & Mallet, J. (1997). The maintenance of species differences across a *Heliconius* hybrid zone. *Heredity , 79,* 495-505.

Jiggins, C. D., & Mallet, J. (2000). Bimodal hybrid zones and speciation. *Tree,* 15 (6): 250-255.

Joger, U., & Bischoff, W. (1983). Zwei neue Taxa der Gattung *Tarentola* (Reptilia: Sauria: Gekkonidae) von den Kanarischen Inseln. *Bonn zool. Beitr.,* 34 (4): 459-468.

Joger, U. (1984a). Taxonomisch Revision der Gattung *Tarentola* (Reptilia: Gekkonidae). *Bonn. Zool. Beitr.,* 35: 129-174.

Joger, U. (1984b). Die Radiation der Gattung *Tarentola* in Makaronesien. *Courier Forsch.-Inst. Senckenberg,* 71: 91-111.

Joger, U. (1984c). Morphologische und biochemisch-immunologische Untersunchungen zur Systematik und Evolution der Gattung *Tarentola* (Reptilia: Gekkonidae). *Zool. Jb. Anat.,* 121: 137-256.

Joger, U., Fritz, U., Guicking, D., Kalyabina-Hauf, S., Nagy, Z. T., & Wink, M. (2007). Phylogeography of western Palaearctic reptiles – Spatial and temporal speciation patterns. *Zoologischer Anzeiger,* 246: 293–313.

Johansson, H., Surget-Groba, Y., & Thorpe, R. S. (2008a). Microsatellite data show evidence for male-biased dispersal in the Caribbean lizard Anolis roquet. *Molecular Ecology,* 17: 4425-4432.

Johansson, H., Surget-Groba, Y., Gow, J. L., & Thorpe, R. S. (2008b). Development of microsatellite markers in the St Lucia anole, *Anolis luciae. Permanent Genetic Resources,* doi:10.1111/j.1755-0998.2008.02206.x.

Johns, G. C., & Avise, J. C. (1998). A comparative summary of genetic distances in the vertebrates from the mitochondrial cytochrome b. *Mol. Biol. Evol.*, 15: 1481-1490.

Johnson, J. B., & Jordan, S. (2000). Phylogenetic divergence in leatherside chub (*Gila copei*) inferred from mitochondrial cytochrome b sequences. *Molecular Ecology,* 9: 1029–1035.

Juan, C., Ibrahim, K. M., Oromí, P., & Hewitt, G. M. (1998). The phylogeography of the darkling beetle, *Hegeter politus*, in the eastern Canary Islands. *Proc R. Soc. Lond. B,* 265: 135-140.

Juan, C., Emerson, B. C., Oromí, P., & Hewitt, G. M. (2000). Colonization and diversification: towards a phylogeographical synthesis for the Canary Islands. *TREE,* 15 (3): 104-109.

Kawakami, T., Butlin, R. K., Adams, M., Saint, K. M., Paull, D. J., & Cooper, S. J. (2007). Differential gene flow of mitochondrial and nuclear DNA markers among chromosomal races of Australian morabine grasshoppers (*Vandiemenella, viatica* species group). *Molecular Ecology,* 16: 5044–5056.

Kim, K. S., & Sappington, T. W. (2004). Boll Weevil (*Anthonomus grandis Boheman*) (Coleoptera: Curculionidae) dispersal in the Southern Uniten states: evidence from mitochondrial DNA variation. *Molecular Ecology and Evolution,* 33 (2): 457-470.

Knowles, L. L., & Richards, C. L. (2005). Importance of genetic drift during Pleistocene divergence as revealed by analyses of genomic variation. *Molecular Ecology,* 14: 4023–4032.

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X., Wilson A. C. (1989). Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. USA,* 86: 6196–6200.

Kruuk, L. E., Baird, S. J., Gale, K. S., & Barton, N. H. (1999b). A comparison of multilocus clines maintained by envoronmental adaptation or by selection against hybrids. *Genetics,* 153: 1959-1971.

Kuchta, S. R. (2007). Contact zones and species limits: hybridization between lineages of the California newt, *Taricha torosa*, in the southern Sierra Nevada. *Herpetologica,* 63 (3): 332–350.

Kvist, L., Broggi, J., Illera, J. C., & Koivula, K. (2005). Colonisation and diversification of the blue tits (*Parus caeruleus teneriffae*-group) in the Canary Islands. *Molecular Phylogenetics and Evolution,* 34: 501–511.

LaDage, L. D., Gutzke, W. H., Simmons II, R. A., & Ferkin, M. H. (2008). Multiple mating increases fecundity, fertility and relative clutch mass in the female leopard gecko (*Eublepharis macularius*). *Ethology,* 114: 512–520.

Lampert, K. P., Rand, A. S., Mueller, U. g., & Ryan, M. J. (2003). Fine-scale genetic pattern and evidence for sex-biased dispersal in the túngara frog, *Physalaemus pustulosus*. *Molecular Ecology,* 12: 3325–3334.

Larmuseau, M. H., Raeymaekers, J. A., Hellemans, B., Van Houdt, J. K., & Volckaert, F. A. (2010). Mitonuclear discordance in the degree of population differentiation in a marine goby. *Heredity,* 105: 532-542.

Larsen, P. A., Marchán-Rivadeneira, M. R., & Baker, R. J. (2010). Natural hybridization generates mammalian lineage with species characteristics. *PNAS,* 107 (25): 11447–11452.

Leaché, A. D., & Cole, C. J. (2007). Hybridization between multiple fence lizard lineages in an ecotone: locally discordant variation in mitochondrial DNA, chromosomes, and morphology. *Molecular Ecology,* 16: 1035–1054.

Leavitt, D. H., Bezy, R. L., Crandall, K. A., & Sites, J. W. (2007). Multi-locus DNA sequence data reveal a history of deep cryptic vicariance and habitat-driven convergence in the desert night lizard *Xantusia vigilis* species complex (Squamata: Xantusiidae). *Molecular Ecology,* 16: 4455–4481.

Lemaire, C., Versini, J. J., & Bonhomme, F. (2005). Maintenance of genetic differentiation across a transition zone in the sea: discordance between nuclear and cytoplasmic markers. *Journal of Evolutionary Biology,* 18 (1): 70-80.

Lewontin, R. C. (1972). The apportionment of human diversity. *Evolutionary Biology,* 6: 381-398.

Lewontin, R. C., & Hartl, D. L. (1991). Population genetics in forensic DNA typing. *Science,* 254: 1745-1750.

Liepelt, S., Kuhlenkamp, V., Anzidei, M., Vendramin, G. G., & Ziegenhagen, B. (2001). Pitfalls in determining size homoplasy of microsatellite loci. *Molecular Ecology Notes,* 1: 332-335.

Lindell, J., Méndez-de la Cruz, F. R., & Murphy, R. W. (2005). Deep genealogical history without population differentiation: Discordance between mtDNA and allozyme divergence in the zebra-tailed lizard (*Callisaurus draconoides*). *Molecular Phylogenetics and Evolution,* 36: 682–694.

Lu, G., Basley, D. J., & Bernatchez, L. (2001). Contrasting patterns of mitochondrial DNA and microsatellite introgressive hybridization between lineages of lake whitefish (*Coregonus clupeaformis*); relevance for speciation. *Molecular Ecology,* 10: 965–985.

Lu, X.-M., Fu, Y.-X., & Zhang, Y.-P. (2002). Evolution of mitochondrial cytochrome b pseudogene in genus *Nycticebus*. *Mol. Biol. Evol.,* 19 (12): 2337–2341.

Lukoschek, V., Waycott, M., & Keogh, J. S. (2008). Relative information content of polymorphic microsatellites and mitochondrial DNA for inferring dispersal and population genetic structure in the olive sea snake, *Aipysurus laevis*. *Molecular Ecology,* 17: 3062–3077.

Lyrholm, T., Leimar, O., Johanneson, B., & Gyllensten, U. (1999). Sex-biased dispersal in sperm whales: contrasting mitochondrial and nuclear genetic structure of global populations. *Proceedings of the Royal Society of London Series B,* 266: 347-354.

MacCallum, C. J., Nüranberger, B., Barton, N. H., & Szymura, J. M. (1998). Habitat Preference in the *Bombina* hybrid zone in Croatia. *Evolution,* 52 (1): 227-239.

Machado, A., López-Jurado, L. F., & Martín, A. (1985). Conservation status of reptiles in the Canary Islands. *Bonn. zool. Beitr.,* 36: 585-606.

Maletzky, A., Kaiser, R., & Mikulíč ek, P. (2010). Conservation genetics of crested newt species *Triturus cristatus* and *T. carnifex* within a contact zone in Central Europe: impact of interspecific introgression and gene flow. *Diversity,* 2: 28-46.

Malhotra, A., & Thorpe, R. S. (1994). Parallels between island lizards suggests selection on mitochondrial DNA and morphology. *Proceedings: Biological Sciences,* 257 (1348): 37-42.

Malhotra, A., & Thorpe, R. S. (2000). The dynamics of natural selection and vicariance in the dominican anole: patterns of within-island molecular and morplohogical divergence. *Evolution,* 54 (1): 245–258.

Mallet, J., McMillan, W. O., & Jiggins, C. D. (1998). Estimating the mating behavior of a pair of hybridizing Heliconius species in the wild. *Evolution,* 52 (2): 503-510.

Mallet, J. (2005). Hybridization as an invasion of the genome. *TRENDS in Ecology and Evolution,* 20 (5): 229-237.

Mallet, J., Beltrán, M., Neukirchen, W., & Linares, M. (2007). Natural hybridization in *heliconiine* butterflies: the species boundary as a continuum. *BMC Evolutionary Biology,* 7 (28): doi:10.1186/1471-2148-7-28.

Martínez-Solano, I., Jockusch, E. L., & Wake, D. B. (2007). Extreme population subdivision throughout a continuous range: phylogeography of *Batrachoseps attenuatus* (Caudata: Plethodontidae) in western North America. *Molecular Ecology,* 16: 4335–4355.

Masta, S. E. (2000). Phylogeography of the jumping spider *Habronattus pugillis* (Araneae: Salticidae): recent vicariance of sky island populations?. *Evolution,* 54 (5): 1699–1711.

Matocq, M. (2002). Morphological and molecular analysis of a contact zone in the *Neotoma fuscipes* species complex. *Journal of Mammalogy,* 83 (3): 866–883.

McGaugh, S. E., Eckerman, C. M., & Janzen, F. J. (2008). Molecular phylogeography of *Apalone spinifera* (Reptilia, Trionychidae). *Zoologica Scripta,* 37 (3): 289–304.

Mehl, K. W., & Schmincke, H.-U. (1999). Structure and emplacement of the Pliocene Roque Nublo debris avalanche deposit, Gran Canaria, Spain. *Journal of Volcanology and Geothermal Research,* 94: 105-134.

Metropolis, N., Rosenbluth, A., Rosenbluth, M., Teller, A., & Teller, E. (1953). Equation of state calculations by fast computing machines. *J. Chem. Phys.,* 21: 1087–1092.

Meyer, A. (1994). Shortcomings of the cytochrome b gene as a molecular marker. *Trends Ecol. Evol.,* 9: 278-280.

Mirol, P. M., Mascheretti, S., & Searle, J. B. (2000). Multiple nuclear pseudogenes of mitochondrial cytochrome b in *Ctenomys* (Caviomorpha, Rodentia) with either great

similarity to or high divergence from the true mitochondrial sequence. *Heredity,* 84 (5): 538–547.

Mishmar, D., Ruiz-Pesini, E., Golik, P., Macaulay, V., Clark, A. G., Hosseini, S., Brandon, M., Easley, K., Chen, E., Brown, M. D., Sukernik, R. I., Olckers, A., & Wallace, D. C. (2003). Natural selection shaped regional mtDNA variation in humans. *PNAS,* 100 (1): 171–176.

Mitchell, P. (1976). Possible molecular mechanisms of the proton motive function of cytochrome systems. *J. theor. Biol.,* 62: 327-367.

Monsen, K. J., & Blouin, M. S. (2003). Genetic structure in a montane ranid frog; restricted gene flow and nuclear-mitochondrial discordance. *Molecular Ecology,* 12: 3275-3286.

Morales, J., Rodríguez, A., Alberto, V., Machado, C., Criado, C., & Rando, J. C. (2009). The impact of human activities on the natural environment of the Canary islands (Spain) during the pre-Hispanic stage (3^{rd}-2^{nd} Century BC to 15^{th} Century AD): an overview. *Environmental Archeology,* 14 (1): 27-31.

Moritz, C., Dowling, T. E., & Brown, W. M. (1987). Evolution of Animal Mitochondrial DNA: Relevance for Population Biology and Systematics. *Annual Review of Ecology and Systematics,* 18: 269-292.

Moritz, C., Schneider, C. J., & Wake, D. B. (1992). Evolutionary relationships within the *Ensatina eschscholtzii* complex confirm the ring species interpretation. *Systematic Biology,* 41: 273-291.

Mossman, C. A., & Waser, P. M. (1999). Genetic detection of sex-biased dispersal. *Molecular Ecology,* 8: 1063–1067.

Muller, H. J. (1942). Isolating mechanisms, evolution, and temperature. *Biol. Symp.,* 6: 71–125.

Munshi-South, J. (2008). Female-biased dispersal and gene flow in a behaviorally monogamous mammal, the large treeshew (*Tupaia tana*). *PLoS ONE,* 3 (9): e3228.

Nei, M., & Roychoudhury, A. K. (1974). Genic variation within and between the three major races of man, Caucasoids, Negroids, and Mongoloids. *Am J Hum Genet,* 26 (4): 421–443.

Nei, M., & Kumar, S. (2000). *Molecular Evolution and Phylogenetics.* New York, USA: Oxford University Press.

Nielsen, E. E., Hansen, M. M., Ruzzante, D. E., Meldrup, D., & Grønkjær, P. (2003). Evidence of a hybrid-zone in Atlantic cod (*Gadus morhua*) in the Baltic and the Danish Belt Sea revealed by individual admixture analysis. *Molecular Ecology,* 12: 1497-1508.

Nielsen, R., & Wakeley, J. (2001). Distinguishing migration from isolation. A Markov chain Monte Carlo approach. *Genetics,* 158: 885–96.

Nogales, M., Lòpez, M., Jiménez-Asensio, J., Larruga, J. M., Hernández, M., & González, P. (1998). Evolution and biogeography of the genus *Tarentola* (Sauria: Gekkonidae) in the Canary Islands, inferred from mitochondrial DNA sequences. *Journal of Evolutionary Biology,* 11: 481-494.

Noor, M. A. (2002). Is the biological species concept showing its age? *Trends in Ecology & Evolution,* 17: 153 –154.

Okamoto, T., & Hikita, T. (2009). Three genetic lineages of the Japanese skink *Plestiodon japonicus* (Scincidae, Squamata) and the genetic composition of their contact zones. *J Zool Syst Evol Res,* 47 (2): 181–188.

Oliver, P. M., Adams, M., Lee, M. S., Hutchinson, M. N., & Doughty, P. (2009). Cryptic diversity in vertebrates: molecular data double estimates of species diversity in a radiation of Australian lizards (Diplodactylus, Gekkota). *Proc. R. Soc. B,* 276: 2001–2007.

Olsson, M., & Shine, R. (2003). Female-biased natal and breeding dispersal in an alpine lizard *Niveoscincus microlepidotus. Biological Journal of the Linnean Society,* 79: 277–83.

Oromí, P., Martín, J. L., Medina, A. L., & Izquierdo, I. (1991). The evolution of the hypogean fauna in the Canary Islands. *The Unity of Evolutionary Biology,* 2: 380-395.

Orr, H. A., & Presgraves, D. C. (2000). Speciation by postzygotic isolation: forces, genes and molecules. *BioEssays,* 22: 1085-1094.

Paine, R T. (1974). Intertidal community structure – experimental studies on the relationship between a dominant competitor and its principal predator. *Oecologia* 15: 93–120.

Palo, J., Varvio, S.-L., Hanski, I., & Väinölä, R. (1995). Developing microsatellite markers for inesct population structure: complex variation in a checkerspot butterfly. *Hereditas,* 123: 295-300.

Payseur, B. A., Krenz, J. G., & Nachman, M. W. (2004). Differential patterns of introgression across the X chromosome in a hybrid zone between two species of house mice. *Evolution,* 58: 2064–2078.

Pedall, I., Fritz, U., Stuckas, H., Valdeon, A., & Wink, M. (2011). Gene flow across secondary contact zones of the *Emys orbicularis* complex in the Western Mediterranean and evidence for extinction and re-introduction of pond turtles on Corsica and Sardinia (Testudines: Emydidae). *J Zool Syst Evol Res* , 49 (1): 44–57.

Pereira, R. J., & Wake, D. B. (2009). Genetic leakage after adaptive and nonadaptive divergence in the *Ensatina eschscholtzii* ring species. *Evolution,* 63 (9): 2288–2301.

Pérez-Torrado, F. J., Carracedo, J. C., & Mangas, J. (1995). Geochronology and stratigraphy of the Roque Nublo Cycle, Gran Canaria, Canary Islands. *Journal of the Geological Society,* 152: 807-818.

Perrin, N., & Mazalov, V. (1999). Dispersal and Inbreeding Avoidance. *The American Naturalist,* 154 (3): 282-292.

Pestano, J., & Brown, R. P. (1999). Geographical structuring of mitochondrial DNA in *Chalcides sexlineatus* within the island of Gran Canaria. *Proceedings of the Royal Society of London Series B-Biological Sciences,* 266: 805-812.

Pestano, J., Brown, R. P., Suárez, N. M., & Fajardo, S. (2003a). Phylogeography of pipistrelle-like bats within the Canary Islands, based on mtDNA sequences. *Molecular Phylogenetics and Evolution,* 26: 56 - 63.

Petit, R. J., & Excoffier, L. (2009). Gene flow and species delimitation. *Trends in Ecology and Evolution,* 24 (7): 386-393.

Phillips, B. L., Baird, S. J., & Moritz, C. (2004). When vicars meet: A narrow contact zone between morphologically cryptic lineages of the rainforest skink, *Carlia rubrigularis*. *Evolution,* 58 (7): 1536-1548.

Piller, K. R., Bart Jr., H. L., & Hurley, D. L. (2008). Phylogeography of the greenside darter complex, *Etheostoma blennioides* (Teleostomi: Percidae): A wide-ranging polytypic taxon. *Molecular Phylogenetics and Evolution,* 46: 974–985.

Pinceel, J., Jordaens, K., Van Houtte, N., De Winter, A.J. & Backeljau, T. (2004). Molecular and morphological data reveal cryptic taxonomic diversity in the terrestrial slug complex *Arion subfuscus/fuscus* (Mollusca, Pulmonata, Arionidae) in continental north-west Europe. *Biol. J. Linn. Soc.,* 83: 23–38.

Pinceel, J., Jordaens, K., & Backeljau, T. (2005). Extreme mtDNA divergences in a terrestrial slug (Gastropoda, Pulmonata, Arionidae): accelerated evolution, allopatric divergence and secondary contact. *J. Evol. Biol.,* 18: 1264-1280.

Posada, D., & Crandall, K. A. (2001). Intraspecific gene genealogies: trees grafting into networks. *TRENDS in Ecology and Evolution,* 16 (1): 37-45.

Posada, D., & Crandall, K. A. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics,* 14 (9): 817-818.

Poulakakis, N., Lymberakis, P., Valakos, E., Pafilis, P., Zouros, E., & Mylonas, M. (2005). Phylogeography of Balkan wall lizard (*Podarcis taurica*) and its relatives inferred from mitochondrial DNA sequences. *Molecular Ecology,* 14: 2433–2443.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics,* 155: 945–959.

Prosser, M. R., Gibbs, H. L., & Weatherhead, P. J. (1999). Microgeographic population genetic structure in the northern water snake, *Nerodia sipedon sipedon* detected using microsatellite DNA loci. *Molecular Ecology,* 8: 329-333.

Pryke, S. R., & Griffith, S. C. (2008). Postzygotic genetic incompatibility between sympatric color morphs. *Evolution,* 63 (3): 793–798.

Quesada, H., Gallagher, C., Skibinski, D. A., & Skibinski, D. O. (1998). Patterns of polymorphism and gene flow of gender-associated mitochondrial DNA lineages in European mussel populations. *Molecular Ecology,* 7 (8): 1041–1051.

Ramel, C. (1997). Mini- and Microsatellites. *Environmental Health Perspectives,* 105 (Supplement 4): 781-789.

Rand, D. M., Haney, R. A., & Fry, A. J. (2004). Cytonuclear coevolution: the genomics of cooperation. *TRENDS in Ecology and Evolution,* 19 (12): 645-653.

Rassmann, K., Tautz, D., Trillmich, F., & Gliddon, C. (1997). The microevolution of the Galápagos marine iguana *Amblyrhynchus cristatus* assessed by nuclear and mitochondrial genetic analyses. *Molecular Ecology,* 6: 452.

Ravaoarimanana, I. B., Teidemann, R., Montagnon, D., & Rumpler, Y. (2004). Molecular and cytogenetic evidence for cryptic speciation within a rare endemic Malagasy lemur, the Northern Sportive Lemur (*Lepilemur septentrionalis*). *Molecular Phylogenetics and Evolution,* 31: 440-448.

Recuero, E., Iraola, A., Rubio, X., Machordom A., & García-París, M. (2007). Mitochondrial differentiation and biogeography of *Hyla meridionalis* (*Anura: Hylidae*): an unusual phylogeographical pattern. *Journal of Biogeography,* 34: 1207-1219.

Redenbach, Z., & Taylor, E. B. (2003). Evidence for bimodal hybrid zones between two species of char (Pisces: Salvelinus), in northwestern North America. *Journal of Evolutionary Biology,* 16: 1135–1148.

Rees, D. J., Emerson, B. C., Oromí, P., & Hewitt, G. M. (2001). Mitochondrial DNA, ecology and morphology: interpreting the phylogeography of the Nesotoes (Coleoptera: Tenebrionidae) of Gran Canaria (Canary Islands). *Molecular Ecology,* 10: 427-434.

Refseth, U. H., Fangan, B. M., & Jakobsen, K. S. (1997). Hybridization capture of microsatellites directly from genomic DNA. *Electrophoresis,* 18: 1519-1523.

Renoult, J. P., Geniez, P., Bacquet, P., Benoit, L., & Crochet, P.-A. (2009). Morphology and nuclear markers reveal extensive mitochondrial introgressions in the Iberian Wall Lizard species complex. *Molecular Ecology,* 18 (20): 4298–4315.

Reyes-Betancort, J. A., Guerra, A. S., Guma, I. R., Humphries, C. J., & Carine, M. A. (2008). Diversity, rarity and the evolution and conservation of the Canary Islands endemic flora. *Anales del Jardín Botánico de Madrid,* 65 (1): 25-45.

Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution,* 43 (1): 223-225.

Rieseberg, L. H. (1998). Molecular ecology of hybridization. In: *Advances in Molecular Ecology (ed. Carvalho, G. R.)*, 243-265. IOS Press, Amsterdam.

Risch, N., Burchard E., Ziv E., & Tang H. (2002). Categorization of humans in biomedical research: genes, race and disease. *Genome Biology,* 3 (7): comment 2007.1–2007.12.

Rivera, P. C., Gardenal, C. N., & Chiaraviglio, M. (2006). Sex-biased dispersal and high levels of gene flow among local populations in the argentine boa contrictor, *Boa contrictor occidentalis*. *Austral Ecology,* 31: 948-955.

Roca, A. L., Georgiadis, N., & O'Brien, S. J. (2005). Cytonuclear genomic dissociation in African elephant species. *Nature Genetics,* 37 (1): 96-100.

Rohwer, S., Bermingham, E., & Wood, C. (2001). Plumage and mitochondrial and haplotype variation across a moving hybrid zone. *Evolution,* 55 (2): 405–422.

Rolán-Alvarez, E., Erlandsson, J., Johannesson, K., & Cruz, R. (1999). Mechanisms of incomplete prezygotic reproductive isolation in an intertidal snail: testing behavioural models in wild populations. *J. Evol. Biol.,* 12: 879-890.

Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *BIOINFORMATICS,* 19 (12): 1572–1574.

Rubidge, E. M., & Taylor, E. B. (2004). Hybrid zone structure and the potential role of selection in hybridizing populations of native west slope cutthroat trout (*Oncorhynchus clarki lewisi*) and introduced rainbow trout (*O. mykiss*). *Molecular Ecology,* 13 (12): 3735-49.

Ruedi, M., Smith, M. F., & Patton, J. L. (1997). Phylogenetic evidence of mitochondrial DNA introgression among pocket gophers in New Mexico (family Geomyidae). *Molecular Ecology,* 6: 453–462.

Ruegg, K. (2007). Genetic, morphological, and ecological characterization of a hybrid zone that spans a migratory divide. *Evolution,* 62 (2): 452-466.

Saint, K. M., Austin, C. C., Donnellan, S. C., & Hutchinson, M. N. (1998). C-mos, a nuclear marker useful for squamate phylogenetic analysis. *Molecular Phylogenetics and Evolution,* 10 (12): 259–263.

Sambrook, J., Fritch, E. F., & Manatis, T. (2000). *Molecular Cloning: A Laboratory Manual* (3 ed., Vol. 3). Cold Spring Harbour, New York, United State of America: Cold SpringHarbour Laboratory Press.

Sarre, S., Smith, G. T., & Meyers, J. A. (1995). Persistence of two species of geckos (*Oedura reticulata* and *Gehyra variegata*) in remnant habitat. *Biological Conservation,* 71: 25-33.

Schmidt, T. R., Wu, W., Goodman, M., & Grossman, L. I. (2001). Evolution of nuclear- and mitochondrial-encoded subunit interaction in cytochrome c oxidase. *Molecular Biology and Evolution,* 18 (4): 563-569.

Schmincke, H.-U., & Sumita, M. (1998). Volcanic evolution of Gran Canaria reconstructed from apron sediments: synthesis of vicap projectdrilling. *Proceedings of the Ocean Drilling Program,* 157: 443-469.

Schneider, C. J., Smith, T. B., Larison, B., & Moritz, C. (1999). A test of alternative models of diversification in tropical rainforests: ecological gradients vs. rainforest refugia. *Proc. Natl. Acad. Sci. USA,* 96: 13869–13873.

Schleich, H. H. (1984). Die Geckos der Gattung *Tarentola* der Kapeverden (Reptilia: Sauria: Gekkonidae). *Courier Forsch.- Inst. Senckenberg,* 68: 95-106.

Selcer, K. W. (1986). Life History of a Successful Colonizer: The Mediterranean gecko, *Hemidactylus turcicus*, in Southern Texas. *Copeia,* 4: 956-962.

Selkoe, K. A., & Toonen, R. J. (2006). Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecology Letters,* 9: 615–629.

Sequeira, F., Alexandrino, J., Rocha, S., Arntzen, J. W., & Ferrand, N. (2005). Genetic exchange across a hybrid zone within the Iberian endemic golden-striped salamander, *Chioglossa lusitanica. Molecular Ecology,* 14: 245–254.

Simonsen, B. T., Siegismund, H. R., & Arctander, P. (1998). Population structure of African buffalo inferred from mtDNA sequences and microsatellite loci: high variation but low differentiation. *Molecular Ecology,* 7: 225–237.

Slatkin, M. (1987). Gene flow and the geographic structure of natural populations. *Science,* 236 (4803): 787-792.

Slatkin, M. (1993). Isolation by distance in equilibrium and non-equilibrium populations. *Evolution,* 47: 264-279.

Smith, C. T., Nelson, R. J., Pollard, S., Rubidge, E., McKay, S. J., Rodzen, J., May., B., & Koop, B. (2002). Population genetic analysis of white sturgeon (*Acipenser transmontanus*) in the Fraser River. *J. Appl. Ichthyol.,* 18: 307–312.

Sotka, E. E., & Palumbi, S. R. (2006). The use of genetic clines to estimate dispersal distances of marine larvae. *Ecology,* 87: 1094–1103.

Sprackland, R. G. & Swinney, G. N. (1998). A new species of giant gecko of the genus *Tarentola* (Reptilia: Squamata: Gekkonidae) from Jamaica. *J. Zool. Lond.,* 46: 73-78.

Steindachner, F. (1891). Über die Reptilien und Batrachier der westlichen und östlichen Gruppe der Kanarischen Inseln. *Ann. naturh. Mus. Wien.,* 6: 287-306.

Stone, K. D., & Cook, J. A. (2000). Phylogeography of black bear (*Ursus americanus*) of the Pacific Northwest. *Can. J. Zool.,* 78: 1218-1223.

Stuart-Fox, D. M., Hugall, A. F., & Moritz, C. (2002). A molecular phylogeny of rainbow skinks (Scincidae: *Carlia*): taxonomic and biogeography implications. *Australian Journal of Zoology,* 50: 39-51.

Suárez, N. M., Bloor, P., Brown, R. P., & Pestano, J. (2008). Highly polymorphic microsatellite loci for the Gran Canarian skink (*Chalcides sexlineatus*) and their applicability in other Canarian Chalcides. *Molecular Ecology Resources,* 8: 666-668.

Suárez, N. M., Betancor, E., Fregel, R., Rodriguez, F., & Pestano, J. (2012). Genetic signature of a severe forest fire on the endangered Gran Canaria blue chaffinch (*Fringilla teydea polatzeki*). *Conservation Genetics*, 13 (2): 499-507.

Sunnucks, P. (2000). Efficient genetic markers for population biology. *TREE,* 15 (5): 199-203.

Sweigart, A. L., Fishman, L., & Willis, J. H. (2006). A simple genetic incompatibility causes hybrid male sterility in *Mimulus. Genetics,* 172: 2465–2479.

Swenson, N. G., & Howard, D. J. (2005). Clustering of contact zones, hybrid zones, and phylogeographic breaks in North America. *The American Naturalist,* 166 (5): 581-591.

Szymura, J. M., & Barton, N. H. (1986). Genetic analysis of a hybrid zone between the fire-bellied toads, *Bombina bombina* and *Bombina variegata* near Cracow in Southern Poland. *Evolution,* 40 (6): 1141-1159.

Szymura, J. M., & Barton, N. H. (1991). The genetic structure of the hybrid zone between the fire-bellied toads *Bombina bombina* and *B. variegata*: comparisons between transects and between loci. *Evolution,* 45 (2): 237-261.

Taberlet, P., & Bouvet, J. (1994). Mitochondrial DNA polymorphism, phylogeography, and conservation genetics of the brown bear (*Ursus arctos*) in Europe. *Proceeding of the Royal Society of London Series B,* 255: 195-200.

Taberlet, P., Swenson, J. E., Sandegren, F., & Bjarvall, A. (1995). Localization of a contact zone between two highly divergent mitochondrial DNA lineages of the brown bear *Ursus arctos* in Scandinavia. *Conservation Biology,* 9 (5): 1255-1261.

Taberlet, P. (1998). Biodiversity at the intraspecific level: The comparative phylogeographic approach. *Journal of Biotechnology,* 64: 91–100.

Taberlet, P., Fumagalli, L., Wust-Saucy, A.-G., & Cosson, J.-F. (1998). Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology,* 7: 453-464.

Tamura, K., & Aotsuka, T. (1988). Rapid isolation method of animal mitochondrial DNA by the alkaline lysis procedure. *Biochemical Genetics,* 26 (11): 815-819.

Tamura, K., Dudley, J., Nei, M., & Kumar, S. (2007). MEGA 4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution,* 24: 1596-1599.

Templeton, A. R. (1998). Nested clade analysis of phylogeographic data: Testing hypotheses about gene flow and population history. *Molecular Ecology,* 7: 381-397.

Thacker, R. W., & Hadfield, M. G. (2000). Mitochondrial phylogeny of extant Hawaiian tree snail (*Achatinellinae*). *Mol. Phyl. Evol.,* 16: 263-270.

Thorpe, R. S. (1991). Clines and cause - microgeographic variation in the Tenerife gecko (*Tarentola delalandii*). *Systematic Zoology,* 40: 172-187.

Thorpe, R. S., & Baez, M. (1993). Geographical variation in scalation of the lizard *Gallotia stehlini* within the island of Gran Canaria. *Biological Journal of the Linnean Society,* 48: 75-87.

Thorpe, R. S., Black, H., & Malhotra, A. (1996). Matrix correspondence tests on the DNA phylogeny of the Tenerife lacertid elucidate both historical causes and morphological adaptation. *Systematic Biology,* 45 (3): 335-343.

Thorpe, R. S., & Malhotra, A. (1996). Molecular and morphologival evolution within small islands. *Philosophical Transactions Royal Society of London series B,* 351: 815-822.

Thorpe, R. S., Surget-Groba, Y., & Johansson, H. (2008). The relative importance of ecology and geographical isolation for speciation in anoles. *Philosophical Transections of the Royal Society B,* 363: 3071-3081.

Thorpe, R. S., Surget-Groba, Y., & Johansson, H. (2010). Genetic tests for ecological and allopatric speciation in Anoles on an Island Archipelago. *PLoS Genet,* 6 (e1000929): doi:10.1371/journal.pgen.1000929.

Thulin, C.-G., & Tegelström, H. (2002). Biased geographical distribution of mitochondrial DNA that passed the species barrier from mountain hares to brown hares (genus *Lepus*): an effect of genetic incompatibility and mating behaviour? *Journal of Zoology,* 258: 299-306.

Toda, M., Hikida, T., Okada, S., & Ota, H. (2003). Contrasting patterns of genetic variation in the two sympatric geckos *Gekko tawaensis* and *G. japonicus* (Reptilia: Squamata) from western Japan, as revealed by allozyme analyses. *Heredity,* 90: 90–97.

Todd, A. C. (2005). The social mating system of *Hoplodactylus maculatus. New Zealand Journal of Zoology,* 32: 251–262.

Tomaru, N., Takahashi, M., Tsumura, Y., Takahashi, M., & Ohba, K. (1998). Intraspecific variation and phylogeographic patterns of *Fagus crenata* (Fagaceae) mitochondrial DNA. *American Journal of Botany,* 85 (5): 629-636.

Toon, A., Hughes, J., Baker, A., & Mather, P. (2003). Discordance between morphology and genetic structure among three plumage forms of the Australian Magpie. *Emu Austral Ornithology,* 103 (4): 337-343.

Townsend, T. M., Larson, A., Louis, E., & Macey, J. R. (2004). Molecular phylogenetics of Squamata: The position of snakes, amphisbaenians, and dibamids, and the root of the Squamate tree. *Syst. Biol.,* 53 (5): 735–757.

Triant, D. A., & DeWoody, J. A. (2007). The occurrence, detection, and avoidance of mitochondrial DNA translocations in mammalian systematics and phylogeography. *Journal of Mammalogy,* 88 (4): 908-920.

Turelli, M., Hoffmann, A. A., & McKechnie, S. W. (1992). Dynamics of cytoplasmic incompatibility and mtDNA variation in natural *Drosophila simulans* Populations. *Genetics,* 132: 713-723.

Ujvari, B., Dowton, M., & Madsen, T. (2008). Population genetic structure, gene flow and sex-biased dispersal in frillneck lizards (*Chlamydosaurus kingii*). *Molecular Ecology,* 17: 3557–3564.

Urquhart, J., Wang, Y., & Fu, J. (2009). Historical vicariance and male-mediated gene flow in the toad-headed lizards *Phrynocephalus przewalskii. Molecular Ecology,* 18, 3714–3729.

Vallianatos, M., Lougheed, S. C., & Boag, P. T. (2001). Phylogeography and genetic characteristics of a putative secondary-contact zone of the loggerhead shrike in central and eastern North America. *Can. J. Zool.,* 79: 2221–2227.

Van Oosterhout, C., Hutchinson, W. F., Wills, D. P., & Shipley, P. (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes,* 4: 535–538.

Vargus, J. M., Guerrero, J. C., & Real, R. (2004). Effects of natural phenomena and human activity on the species richness of endemic and non-endemic Heteroptera in the Canary islands. *Animal Biodiversity and Conservation,* 27 (2): 57-66.

Vitalis, R. (2002). Sex-specific genetic differentiation and coalescence times: estimating sex-biased dispersal rates. *Molecular Ecology,* 11: 125-138.

Vitousek, P. M., D'Antonio, C. M., Loope, L. L., Rejmánek, M., & Westbrooks, R. (1997). Introduced species: A significant component of human-caused global change. *New Zealand Journal of Ecology,* 21 (1): 1-16 .

Vogel, P., Cosson, J.-F., & Jurado, L. F. (2003). Taxonomic status and origin of the shrews (*Soricidae*) from the Canary islands inferred from a mtDNA comparison with the European *Crocidura* species. *Molecular Phylogenetics and Evolution,* 27 (2): 271-282.

Wahlund, S. (1928). Zusammensetzung von Population und Korrelationserscheinung vom Standpunkt der Vererbungslehre aus betrachtet. *Hereditas,* 11: 65–106.

Waits, L., Taberlet, P., Swenson, J. E., Sandegren, F., & Franzen, R. (2000). Nuclear DNA microsatellite analysis of genetic diversity and gene flow in the Scandinavian brown bear (*Ursus arctos*). *Molecular Ecology,* 9: 421-431.

Wake, D. B. (1997). Incipient species formation in salamanders of the *Ensatina* complex. *Proceedings of the National Academy of Sciences,* 94: 7761–7767.

Wattier, R., Engel, C. R., Saumitou-Laprade, P., & Valero, M. (1998). Short allele dominance as a source of heterozygote deficiency at microsatellite loci: experimental evidence at the dinucleotide locus Gv1CT in *Gracilaria gracilis* (Rhodophyta). *Molecular Ecology,* 7: 1569-1573.

Widmer, A., Schmid-Hempel, P., Estoup, A., & Scholl, A. (1998). Population genetic structure and colonization history of *Bombus terrestris* s.l. (Hymennoptera: Apidae) from the Canary Islands and Madeira. *Heredity,* 81: 563-572.

Wiens, J. J., & Penkrot, T. A. (2002). Delimiting species using DNA and morphological variation and discordant species limits in spiny lizards (*Sceloporus*). *Syst. Biol.,* 51 (1): 69–91.

Wilkins, J. F., & Marlowe, F. W. (2006). Sex-biased migration in humans: what should we expect from genetic data? *BioEssays,* 28 (3): 290-300.

Wolf, J. B., Harrod, C., Brunner, S., Salazar, S., Trillmich, F., & Tautz, D. (2008). Tracing early stages of species differentiation: Ecological, morphological and genetic divergence of Galápagos sea lion populations. *BMC Evolutionary Biology,* 8 (150): doi:10.1186/1471-2148-8-150.

Wolff, J. O., & Plissner, J. (1998). Sex biased in avian natal dispersal: an extension of the mammalian model. *Oikos,* 83: 327–30.

Wright, S. (1943). Isolation by distance. *Genetics,* 28: 114-138.

Yan, J., Wang, Q., Chang, Q., Ji, X., & Zhou, K. (2010). The divergence of two independent lineages of an endemic Chinese gecko, *Gekko swinhonis*, launched by the Qinling orogenic belt. *Molecular Ecology,* 19 (12): 2490–2500.

Yang, D. S., & Kenagy, G. (2011). Population delimitation across contrasting evolutionary clines in deer mice (*Peromyscus maniculatus*). *Ecology and Evolution,* Early view.

Yang, Z., & Rannala, B. (2010). Bayesian species delimitation using multilocus sequence data. *PNAS,* 107 (20): 9264–9269.

Zarza, E., Reynoso, V. H., & Emerson, B. C. (2008). Diversification in the northern neotropics: mitochondrial and nuclear DNA phylogeography of the iguana *Ctenosaura pectinata* and related species. *Molecular Ecology,* 17: 3259–3275.

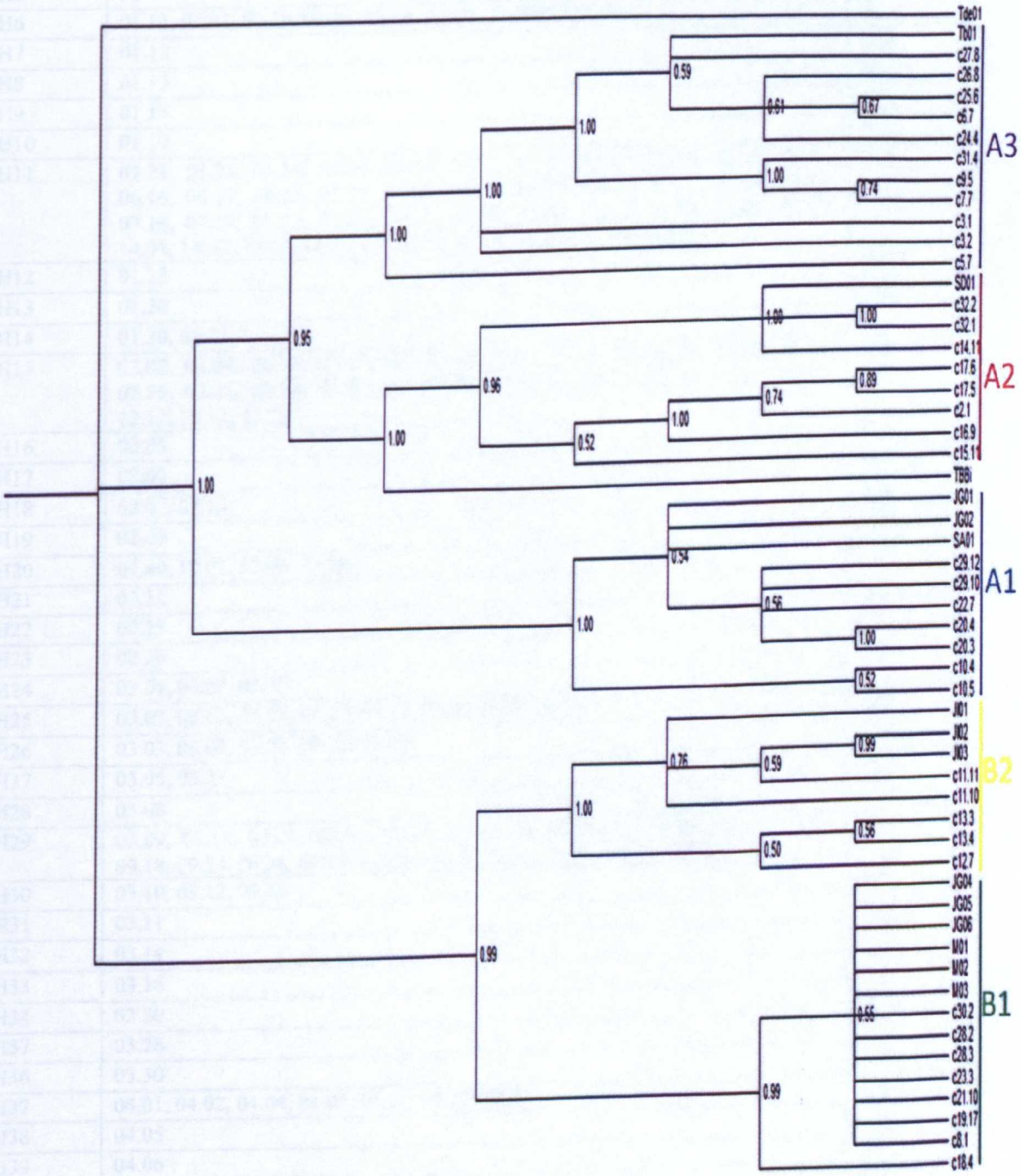
Zarza, E., Reynoso, V. H., & Emerson, B. C. (2011). Discordant patterns of geographic variation between mitochondrial and microsatellite markers in the Mexican black iguana (*Ctenosaura pectinata*) in a contact zone. *Journal of Biogeography,* 38: 1394–1405.

Zhang, D.-X., & Hewitt, G. M. (2003). Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. *Molecular Ecology,* 12: 563-584.

Zhang, Z., Huang, L., Shulmeister, V. M., Chi, Y.-I., Kim, K. K., Hung, L.-W., Crofts, A. R., Berry, E. A., & Kim, S.-H. (1998). Electron transfer by domain movement in cytochrome bc1. *Nature,* 392: 677-684.

Appendices

Appendix 1 A reference tree constructed from 13 *T. boettgeri* cytb sequences obtained in this study and 39 sequences from Gübitz et al. (2005) (marked with 'c') with *T. delalandii* (Tdel01) as an outgroup. Numbers on branches are Bayesian posterior probabilities.



Appendix 2 List of haplotypes

Haplotype	Individual	Lineage
H1	01.01, 01.07, 01.10, 01.17, 01.20, 04.23, 04.25, 04.27, 11.04, 11.07, 11.08, 11.18, 11.29, OG2, OG3, OG5	B1b
H2	01.02, 01.04, 01.06	B2
H3	01.03, 01.09, 01.14, 01.16, 01.25	B1a
H4	01.05	A1a
H5	01.08	B1a
H6	01.11, 02.01, 02.10, 02.13, 02.14, 02.19, 02.29, 03.13, 03.21	A1a
H7	01.12	B1a
H8	01.13	B2
H9	01.18	B1a
H10	01.19	B1a
H11	01.21, 01.23, 01.24, 04.03, 04.12, 04.22, 05.01, 05.04, 06.03, 06.14, 06.15, 06.16, 06.17, 06.18, 06.20, 06.21, 06.23, 06.24, 06.30, 07.03, 07.04, 07.11, 07.16, 07.19, 07.22, 07.28, 07.31, 10.01, 10.02, 10.14, 10.28, 11.05, 11.10, 14.01, 14.02, 14.03, 14.07, 14.08, 14.12, 14.14, 14.15, JG04, JG05	A1a
H12	01.22	B2
H13	01.26	A1a
H14	01.30, 01.31	B1a
H15	02.02, 02.04, 02.05, 02.15, 02.16, 02.17, 02.18, 02.20, 02.21, 02.22, 02.24, 02.25, 02.27, 02.28, 02.30, 03.15, 03.17, 03.19, 03.25, 03.26, 03.27, 09.03, 12.12, 13.12, 13.15	A1a
H16	02.03	A1a
H17	02.06	A1a
H18	02.07, 02.12	A1a
H19	02.08	A1b
H20	02.09, 12.01, 12.03, 12.04	A1a
H21	02.11	A1a
H22	02.23	A1a
H23	02.26	A1a
H24	03.01, 03.07, 03.16	A1a
H25	03.02, 08.02, 08.07, 08.16, 08.18, 09.01, 09.10, 09.20	A1a
H26	03.03, 03.04, 03.06, 03.22, 03.29	A1a
H27	03.05, 03.23	A1a
H28	03.08	A1a
H29	03.09, 07.15, 07.20, 08.08, 08.15, 08.17, 08.19, 08.25, 08.28, 09.02, 09.05, 09.18, 09.24, 09.28, 09.29, 12.05, 12.16, 12.20, 12.21, 12.28, 13.11	A1a
H30	03.10, 03.12, 03.24	A1a
H31	03.11	A1a
H32	03.14	A1a
H33	03.18	A2
H34	03.20	A1a
H37	03.28	A1a
H36	03.30	A1a
H37	04.01, 04.02, 04.04, 04.07, 04.21, 04.28, M02	B1a
H38	04.05	B1a
H39	04.06	B1a
H40	04.08, 06.04, 07.27	B1a

Haplotype	Individual	Lineage
H41	04.09, 04.14, 04.18, 04.24, 04.31, 05.02, 05.03, 05.05, 05.06, 05.12, 05.16, 05.18, 05.19, 05.21, 05.22, 05.24, 05.25, 05.27, 05.29, 05.30, 05.31, 06.01, AC1, AC2, AC3, OG4	B1a
H42	04.10	B1a
H43	04.11, 04.17, 04.19, 04.26, 04.30	B1a
H44	04.13	B1a
H45	04.15	B1b
H46	04.16, 11.02	B1b
H47	04.20	B1a
H48	04.29	B1a
H49	05.07	B1a
H50	05.09, 05.11	B1b
H51	05.10	B1a
H52	05.13	B1a
H53	05.14	B1a
H54	05.17	B1a
H55	05.20	B1a
H56	05.26	B1a
H57	05.28	B1a
H58	06.02, 06.25, 06.26	B1a
H59	06.05	B1a
H60	06.06	B1a
H61	06.07, 06.29	B1a
H62	06.08	B1a
H63	06.09, 06.22, 10.05, 10.26	B1a
H64	06.10, 06.19, 07.05, 14.04	B1a
H65	06.11	B1a
H66	06.12, 06.27	B2
H67	06.13	B1a
H68	06.28	B1a
H69	07.01, 07.06	A1a
H70	07.02	A1a
H71	07.07	B1a
H72	07.09	A1a
H73	07.10, JG06	A1a
H74	07.14, 08.01, JG01, SA01	A1a
H75	07.17, 07.29	B1a
H76	07.18, 07.26	B1a
H77	07.23	B1a
H78	07.25	B1a
H79	07.30	B1a
H80	08.03, 08.23	A1a
H81	08.04	A1a
H82	08.05	A1a
H83	08.06	A1a
H84	08.09	A1a
H85	08.10	A1a
H86	08.11, 08.30	A1a
H87	08.12	A1a
H88	08.13	A1a
H89	08.20	A1a

Haplotype	Individual	Lineage
H90	08.21	A1a
H91	08.22	A1a
H92	08.26	A1a
H93	08.29	A1a
H94	09.04	A1a
H95	09.08	A1a
H96	09.13, 13.02, 13.07, 13.10, 13.17	A1a
H97	09.14, 09.19	A1a
H98	09.15	A1a
H99	09.17	A1a
H100	09.21	A1a
H101	09.22	A1a
H102	09.23	A1a
H103	09.26	A1a
H104	09.27	A1a
H105	09.30	A1a
H106	10.03	B1b
H107	10.04, 10.21	B1a
H108	10.06	B1a
H109	10.08	B1a
H110	10.09	B1a
H111	10.10	B1a
H112	10.11	B1a
H113	10.16	B1a
H114	10.17	B1a
H115	10.18	B1a
H116	10.19	B1a
H117	10.20	B1a
H118	10.22	B1a
H119	10.23, 11.20	B1a
H120	10.24	B1a
H121	10.25	B1a
H122	10.27	B1a
H123	10.29	B1a
H124	10.30	B1a
H125	11.01, 11.25, 11.26, 11.27, 11.28, M03	B1a
H126	11.03, 11.23, 11.24	B1b
H127	11.06, 11.09	B1a
H128	11.11	B1a
H129	11.12	B1b
H130	11.13	B1b
H131	11.14	B1b
H132	11.16	B1b
H133	11.17	B1b
H134	11.19	B1b
H135	11.21	B1b
H136	11.22	B1b
H137	11.30	B1b
H138	12.02	A1a
H139	12.06	A1b

Haplotype	Individual	Lineage
H140	12.08	A1a
H141	12.09	A1a
H142	12.10	A1a
H143	12.11, 12.18, 12.19	A1b
H144	12.13	A1a
H145	12.22	A1a
H146	12.23	A1a
H147	12.24	A1b
H148	12.25	A1a
H149	12.26	A1b
H150	12.27	A1b
H151	12.29	A1a
H152	12.30	A1a
H153	13.01	A1a
H154	13.03	A1a
H155	13.04	A1a
H156	13.05	A1a
H157	13.06	A1b
H158	13.08	A1b
H159	13.09	A1a
H160	13.13	A1a
H161	13.14	A1a
H162	13.16	A1a
H163	14.05	B1a
H164	14.06	B1a
H165	14.09	B1a
H166	AC4	B1b
H167	EC1	B1b
H168	JG02	A1a
H169	JI01, JI03	B2
H170	JI02	B2
H171	LS1	B2
H172	LS2, LS3	B1a
H173	M01	B1a
H174	OG1	B1a
H175	SD01	A2
H176	Tb01	A3

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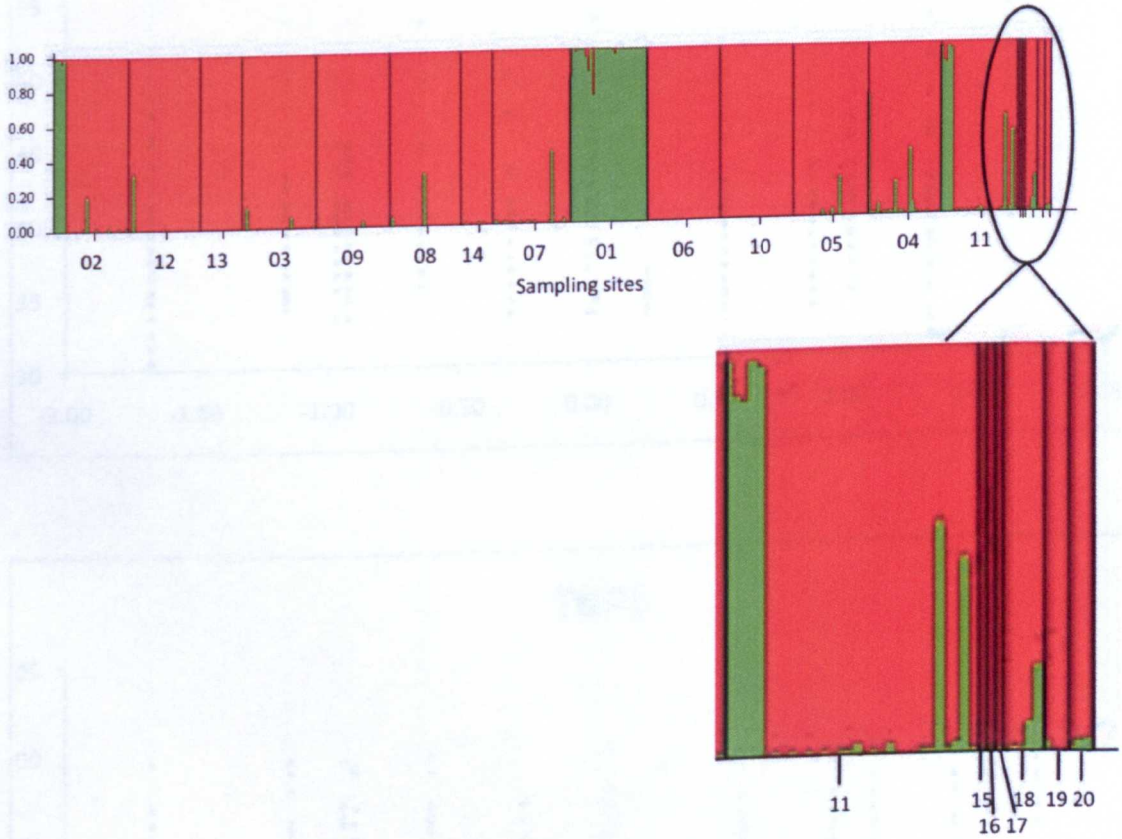
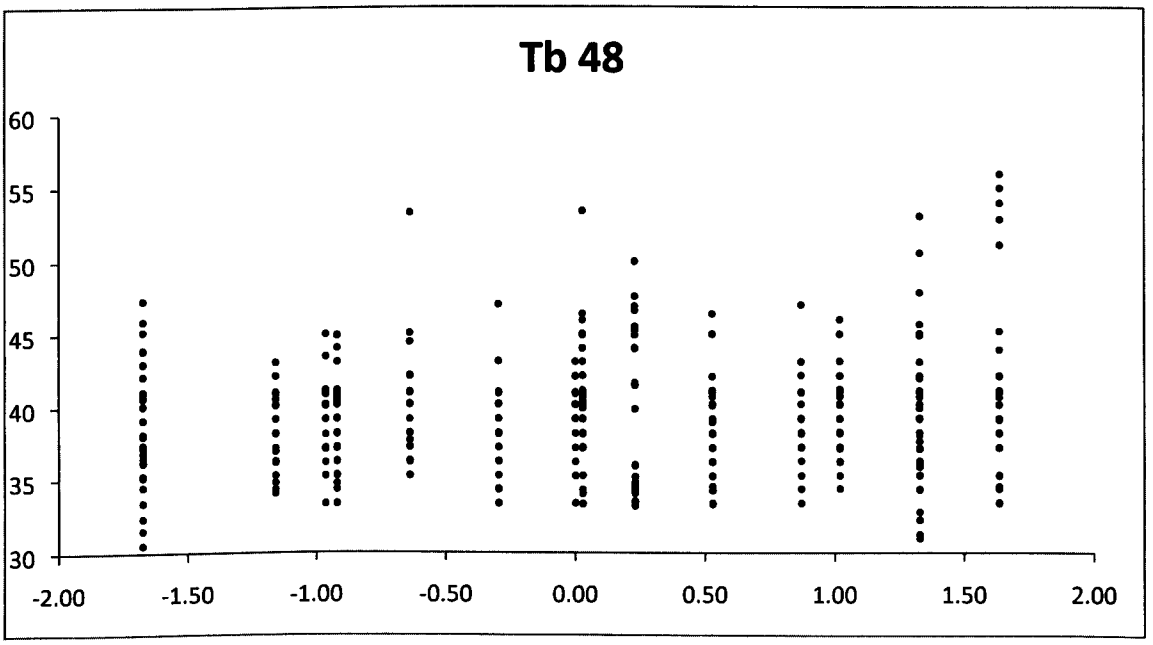
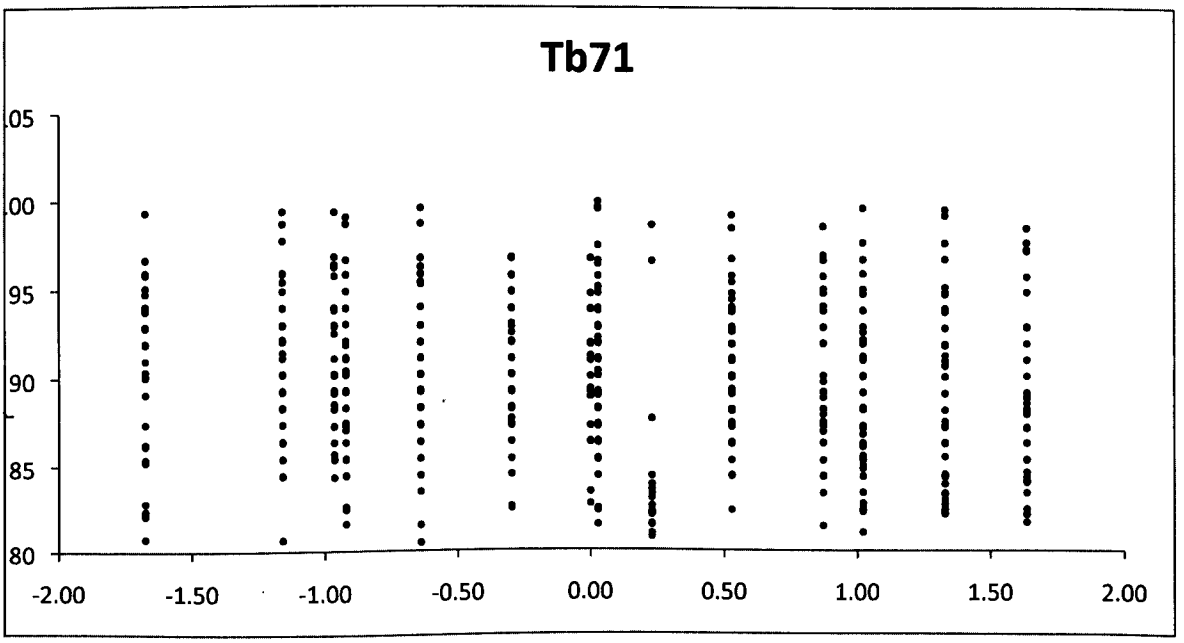
Appendix 4 Assessment of population structure based on ten microsatellite loci and assuming two populations (k = 2). The y-axis represents proportions of the multilocus genotype in each site that is attributable to co-ancestry with cluster 1 (red) and 2 (green). Individuals are grouped along the x-axis according to their sites. This analyses differs from Figure 26 because individuals sampled from site SA (15), Tb (16), SD (17), JG (18), M (19) and JI (20) were included here. Table shows proportion of populations in each genetic cluster for site SA, Tb, SD, JG, M and JI.

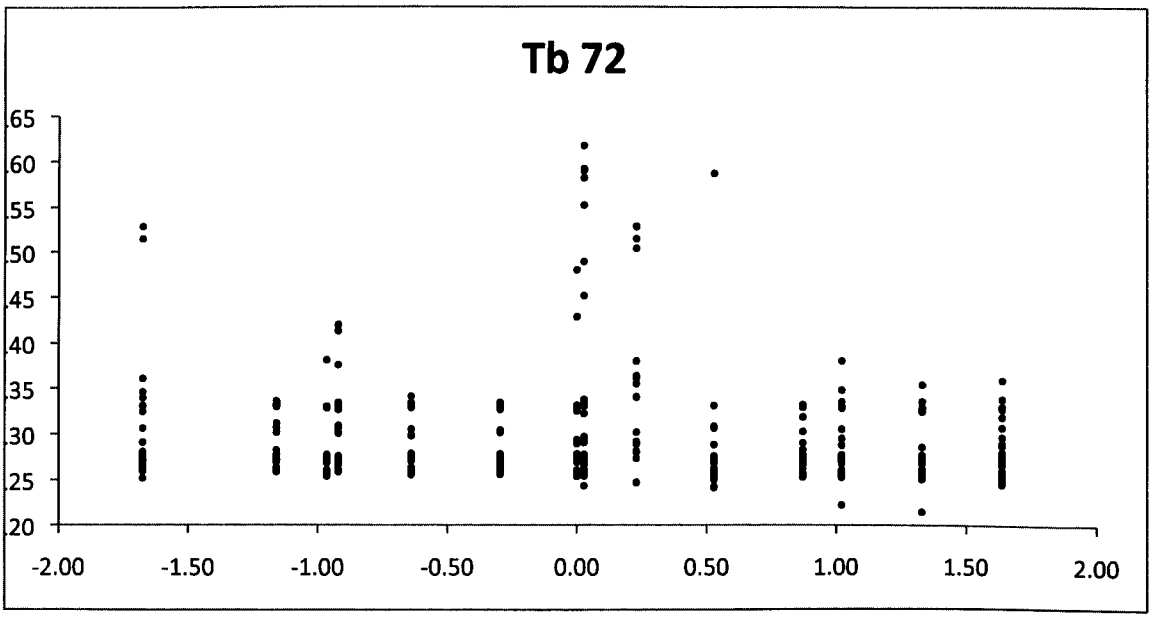


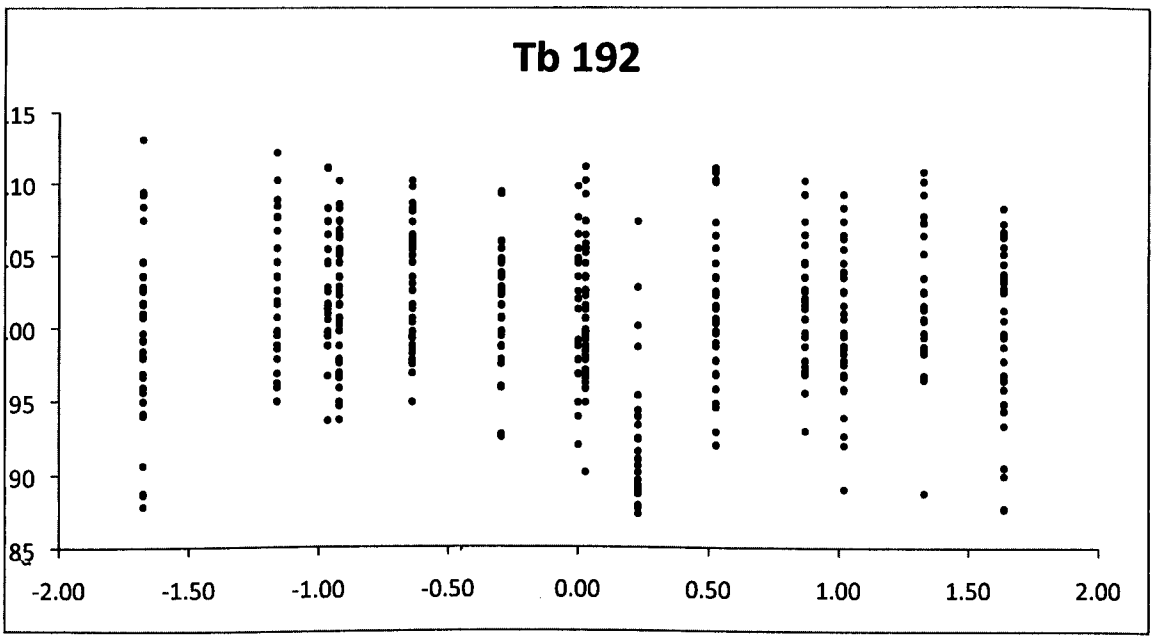
Site	Inferred Clusters	
	1	2
SA	0.995*	0.005
Tb	0.995*	0.005
SD	0.990*	0.010
JG	0.937*	0.063
M	0.988*	0.012
JI	0.978*	0.022

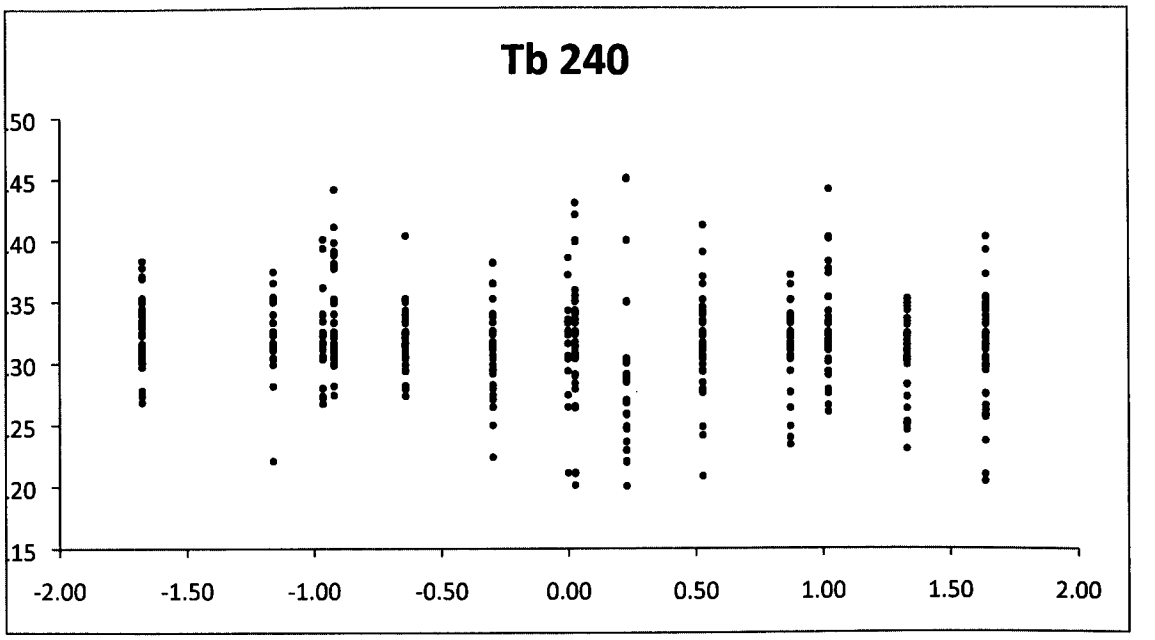
Appendix 5 Dot plots of genotype scores of each microsatellite locus against putative geographical locality. Centre of the SE transect represented as '0'.

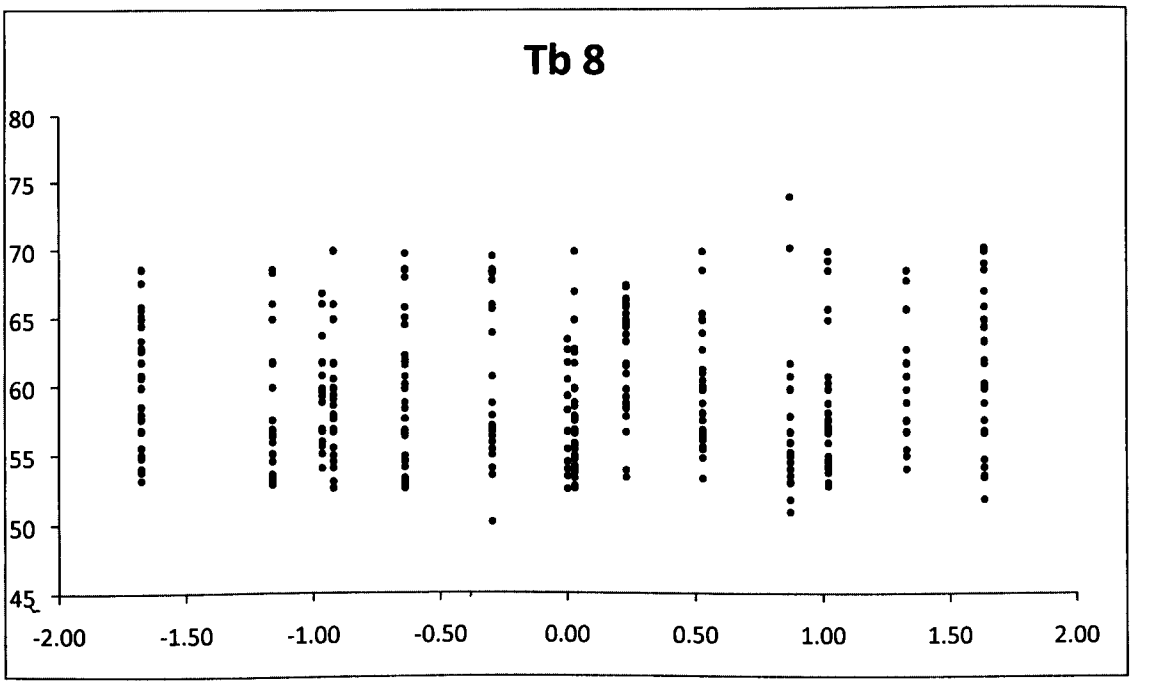


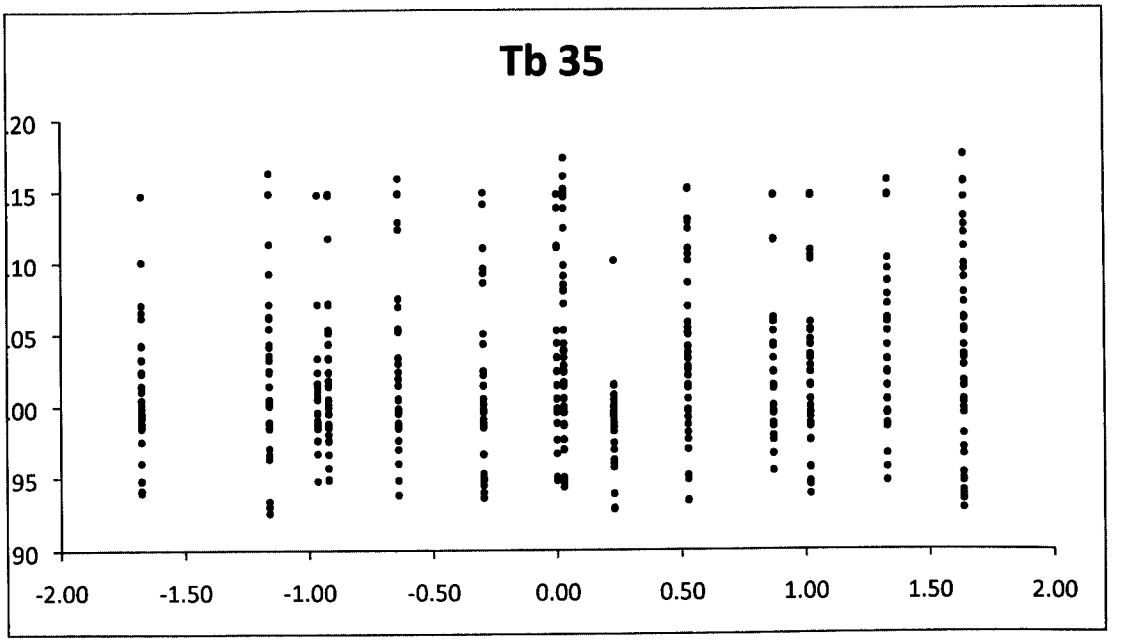


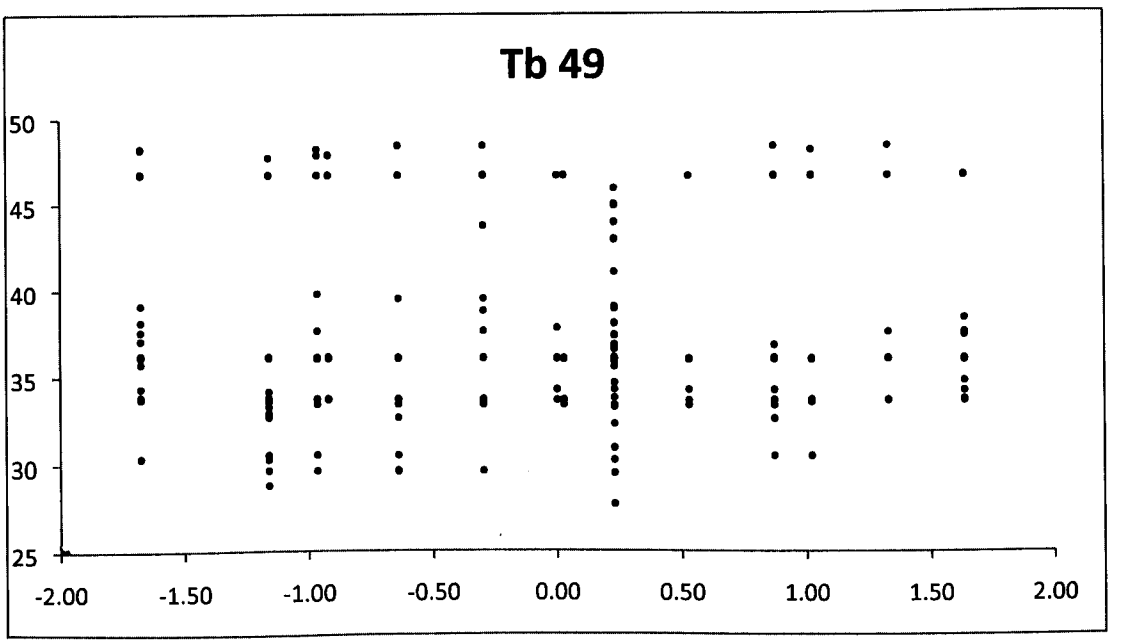

Tb 72
.65
.60
.55
.50
.45
.40
.35
.30
.25
.20
-2.00
-1.50
-1.00
-0.50
0.00
0.50
1.00
1.50
2.00

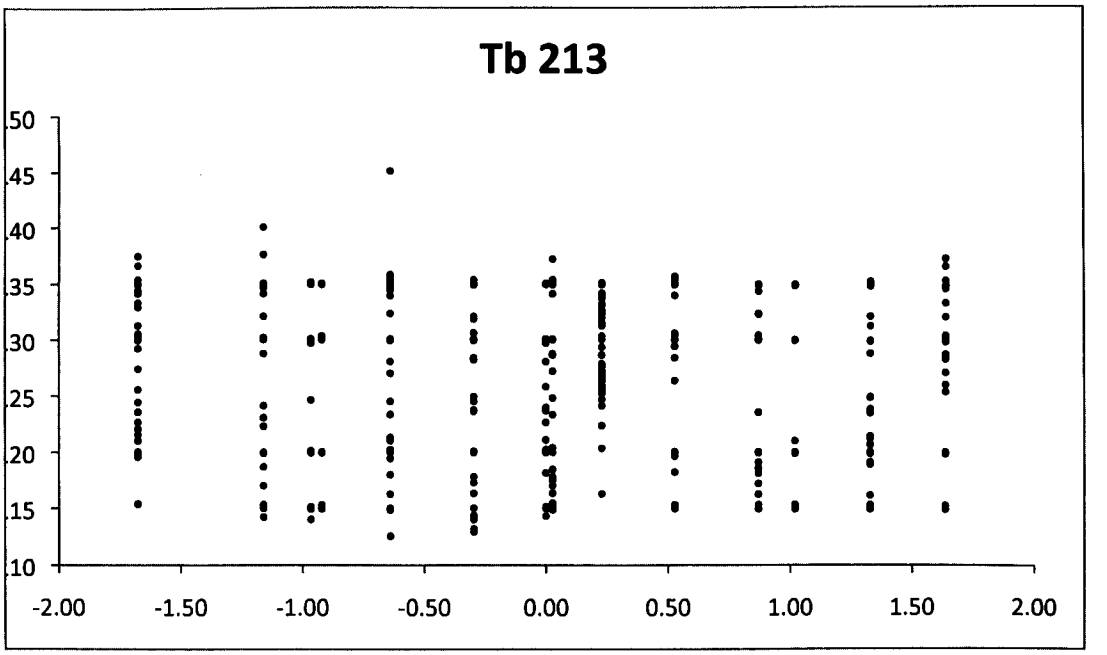

Tb 192
.15
.10
.05
.00
.95
.90
.85
-2.00
-1.50
-1.00
-0.50
0.00
0.50
1.00
1.50
2.00

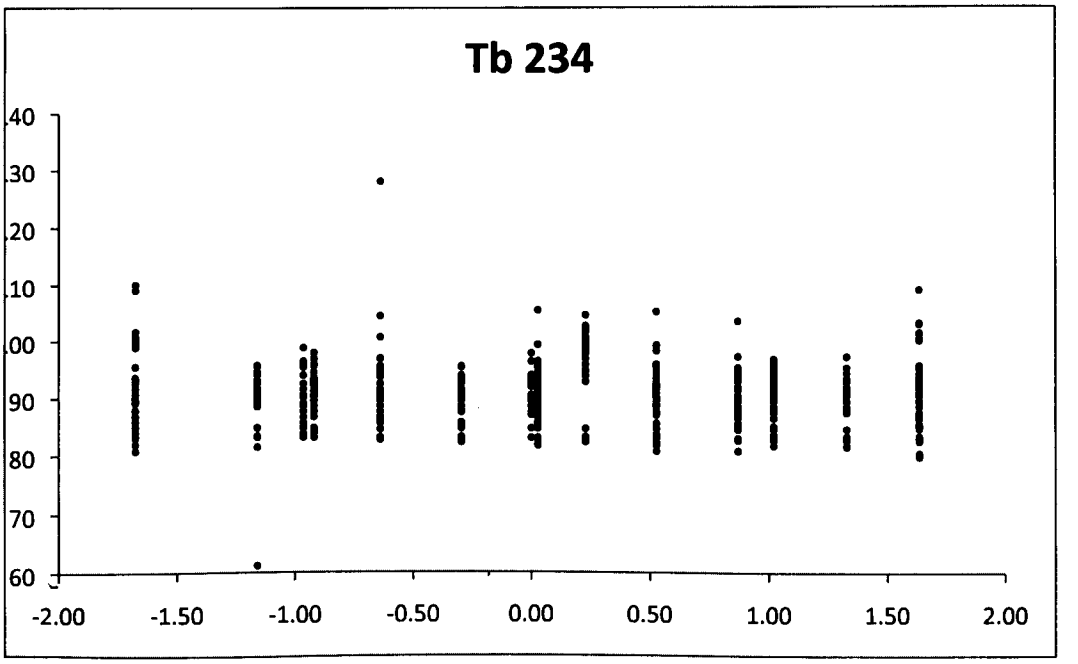

Tb 240
.50
.45
.40
.35
.30
.25
.20
.15
-2.00
-1.50
-1.00
-0.50
0.00
0.50
1.00
1.50
2.00


Tb 8
80
75
70
65
60
55
50
45
-2.00
-1.50
-1.00
-0.50
0.00
0.50
1.00
1.50
2.00


Tb 35
20
15
10
05
00
95
90
-2.00
-1.50
-1.00
-0.50
0.00
0.50
1.00
1.50
2.00


Tb 49
50
45
40
35
30
25
-2.00
-1.50
-1.00
-0.50
0.00
0.50
1.00
1.50
2.00


Tb 213
50
45
40
35
30
25
20
15
10
-2.00
-1.50
-1.00
-0.50
0.00
0.50
1.00
1.50
2.00


Tb 234
40
30
20
10
00
90
80
70
60
-2.00
-1.50
-1.00
-0.50
0.00
0.50
1.00
1.50
2.00