



The potential impact of climate change on the genetic diversity of the endangered western leopard toad, *Sclerophrys pantherina*



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Abstract

Climate change is now considered to be one of the greatest threats to the persistence of biodiversity. Much work has focused on the potential for climatic shifts to alter species' ranges, phenology, physiology, and behaviour, addressing higher level units of biodiversity from populations to biomes. However, the potential effects of climate change on the most fundamental unit of biodiversity, intraspecific genetic diversity, has only recently received research attention. Studies to date suggest that the accelerated climatic changes we currently face could cause a loss of intraspecific diversity, hampering the ability of populations to respond to further environmental change.

Amphibians are considered to be one of the most vulnerable taxa to climate change. The amphibians of the Western Cape of South Africa provide a powerful opportunity to study the impact of climate change on genetic diversity, as many are endemic, threatened, and generally considered to be poor dispersers, limiting their ability to respond to climatic changes through range shifts. This project had two aims: first, to explore the potential impact of climatic shifts on the genetic landscape of the endemic and Endangered western leopard toad, *Sclerophrys pantherina*, a species with a disjunct distribution on either side of the Cape Flats. Second, I aimed to test the hypothesis that climatic fluctuations drive genetic divergence, a mechanism which may explain the potential overlap of high diversity areas with areas of high climatic instability.

Population genetic analyses supported the findings of previous genetic work on *S. pantherina*, that populations in the Cape Metropole and the Overstrand Municipality (to the west and east of the Cape Flats, respectively) are genetically distinct, and thus should be treated as separate conservation units. Higher haplotype diversity was identified in the populations in the Cape Metropole when compared with the Overstrand, highlighting the importance of urban habitat patches in harbouring diversity in the species. Distinct pockets of low haplotype diversity were identified at Observatory and Hout Bay, suggesting a lack of connectivity between these and adjacent breeding sites, likely due to urban-associated habitat fragmentation. Species distribution modelling revealed that the species could lose a substantial amount of climatically suitable space in its current area of occurrence by 2070. Furthermore, the degree of loss was not uniform across the species' distribution. The populations of the Cape Metropole were predicted to experience greater losses in climatically suitable space than populations in the Overstrand. Additionally, the change in climatic suitability between the mid-Holocene (~6,000

years ago) and present as well as the change in suitability between future (2050 and 2070) and present were significant predictors of genetic diversity, where areas of the greatest change in suitability between time periods were associated with the highest genetic diversity. Future efforts to conserve the species should focus on establishing connectivity between breeding sites to allow for the rescue of genetically depauperate sites. Efforts to mitigate the drastic negative effects of climate change predicted by the species distribution models should prioritise the breeding sites in the Cape Metropole, which are both higher in diversity and at greater risk from climate change. Mitigation efforts will likely require the application of engineered solutions to promote the maintenance of suitable wetland habitat for the species.

1. Introduction

1.1. Effects of climate change on biodiversity

1.1.1. Projections and accelerating rates of change

It is now beyond debate in the scientific community that the climate is changing more rapidly than ever, with the contemporary rate of global atmospheric warming greatly exceeding the natural variability of the past 1,000 years (Crowley 2000). The latest report of the Intergovernmental Panel on Climate Change (IPCC) gives a clear indication that the global mean temperature is warming and patterns of precipitation across the globe are changing. Between 1880 and 2012, the average global mean temperature increased by 0.85°C, and the Earth is continuing to warm at an accelerated rate (IPCC 2013). By 2100, temperature is expected to increase by another 1.5–2.0°C. Precipitation patterns have also changed, but in a much more varied way. Over the next century, precipitation is likely to increase in wet regions at mid latitudes, and decrease in dry subtropical regions and dry mid latitudes, particularly in regions with Mediterranean climates (IPCC 2013). An increase in the frequency of extreme events, including warm days and heavy precipitation events, is also predicted to occur in the next few decades.

These climate changes are attributable to increased radiative forcing, primarily due to an increase in the concentration of greenhouse gases, mainly carbon dioxide (CO₂), in the atmosphere, caused by human fossil fuel emissions and land-use change (Hegerl et al. 2007; Forster et al. 2007). Atmospheric greenhouse gas concentrations have increased to unprecedented levels, with current CO₂ concentrations 40% higher than pre-industrial levels (IPCC 2013). Moreover, it is estimated that these changes to the climate system are largely irreversible, at least for 1,000 years after carbon emissions are eliminated completely (Solomon et al. 2009).

1.1.2. Climate-induced range shifts

Much work has been done across many regions of the globe and across many taxa, investigating the effect of these climatic changes on biodiversity, with some authors predicting that climate change could surpass habitat destruction as the greatest global threat to biodiversity in the next few decades (Leadley et al. 2010). Furthermore, unlike some other drivers of biodiversity loss, the effects of climate change are varied, complex, and, in some cases, only observable by long-

term observation. In a recent review, the response of biodiversity to climate change was defined on the basis of three axes: spatial, temporal, and “self” (Bellard et al. 2012). This categorization reflects the multi-faceted nature of climate change impacts, which can include loss of climatically suitable space, changes in the timing of key life history events, and also physiological or behavioural alterations, referred to as the “self” axis. Most studies to date have focused solely on the spatial axis of the climate change response, assessing to what extent species’ ranges are expected to shift under future climate scenarios. Although it can be difficult to assign causation of biological phenomena to climate change, rather than other more short-term drivers of change, long-term trends in species’ range shifts have been found to be “heavily biased in the directions predicted from global warming” (Parmesan 2006). For example, a global meta-analysis looking at approximately 100 species of birds, butterflies, and plants primarily from the northern hemisphere found that, in accordance with climate change predictions, species’ ranges have shifted an average of 6 km per decade towards the poles, and to higher elevations at an average rate of 6 m per decade (Parmesan & Yohe 2003). Another meta-analysis of a diverse range of North American taxa found that 275 species have experienced northward range shifts over a range of time periods during the 20th century, correlating with recent climatic shifts (Hickling et al. 2006). Here an average northward shift of 30-60 km was identified. The same study found significant elevational shifts in over 200 species, with the majority of species moving to higher elevations, in accordance with expectations corresponding to recent climate change. This finding is further supported by a subsequent meta-analysis, including some southern hemisphere species, which reported shifts to higher latitudes at a median rate of approximately 17 km per decade, and to higher elevations at a median rate of 11 m per decade, nearly double the estimates from previous meta-analyses (Chen et al. 2011). Moreover, due to the interspecific variability in rates of range shifting, heterogeneous climatic shifts across a region can create novel species assemblages and, potentially, altered community dynamics (Walther et al. 2002; Walther 2010).

1.1.3. Effects of climate change on species’ phenologies

As previously mentioned, the effects of climate change on biodiversity go far beyond latitudinal or altitudinal shifts in species distributions, and can include changes in a species’ phenology, the annual timing of key life history events such as emergence from hibernation or the start of breeding (see Parmesan 2006 for an extensive review on this topic.) A particularly striking example has been identified in the temperate marine realm, where seasonal production

in many planktonic species has significantly advanced over the period from 1958 to 2002, correlating with increased spring sea surface temperatures (Edwards & Richardson 2004). As expected, in a system where recruitment in many species at higher trophic levels is carefully timed to match peak planktonic production, these changes have had marked community-level consequences. In the terrestrial environment, one of the first indications of phenological change has come from farmers' observations of a lengthening vegetative growing season (Parmesan 2006). Observations of experimental growing sites across Europe have supported these anecdotes, with an advancement of leaf unfolding by an average of 6.3 days and a delay of autumn leaf colouration by an average of 4.5 days reported between 1959 and 1996 (Menzel 2000). Because the progression of the growing season, and thus harvest dates, are explained primarily by spring temperatures, it is highly likely that this advancement is due to atmospheric warming (Menzel et al. 2003). As for animals, many studies to date have focused on butterflies and birds, with dates of first emergence and laying dates advancing in parallel with warming spring temperatures (Forister & Shapiro 2003; Both et al. 2004). Due to the intimate symbioses, particularly multi-trophic level interactions, that exist within communities, phenological changes at one trophic-level can have ripple effects on other community members, altering the structure and function of whole ecological networks (Visser & Both 2005; Walther 2010).

1.1.4. Climate change and species extinctions

Many authors have attempted to quantify species losses under projected future climate change scenarios, with species loss frequently calculated as a function of total habitat area lost or climate-induced range contraction (Bellard et al. 2012). For example, using this approach, Thomas et al. (2004) predicted that under mid-range warming scenarios for 2050, 15-37% of species considered would be "committed to extinction." Other authors have projected future distributions of major vegetation types in a higher CO₂ environment using biome area loss as a proxy to quantify losses of endemic plant and vertebrate species from biodiversity hotspots (Malcolm et al. 2006). Although these results were highly dependent on *a priori* assumptions of the biome specificity of species, it was found that an average of 12%, (and up to 43%) of the endemic biota of biodiversity hotspots could become extinct if CO₂ doubles in the next 100 years, with species loss resulting from climate change exceeding that from deforestation in some tropical hotspots. Among those hotspots expected to be most affected are the Cape Floristic Region, the Caribbean, and the Tropical Andes (Malcolm et al. 2006).

As dire as these projections may seem, few global species extinctions to date have been attributed primarily to contemporary climate change, with only 20 out of 864 extinctions reported by the IUCN linked to anthropogenic climate change (Cahill et al. 2012). However, contractions at the warm edge of a species range have frequently been cited as the cause of local extinctions, and climate change has been identified as the proximate cause in some cases of local extinction and population decline. For example, although it is globally considered a species of Least Concern (Smith & Beever 2016), in the American pika *Ochotona princeps* there has been a five-fold increase in the rate of upslope range contraction and the rate of local extinction from 1898–2008, with data collected from habitat temperature sensors implicating climatic stress as the cause of extirpation from many previously occupied sites (Beever et al. 2010, 2011). However, more often than not climate change does not cause extinction purely by overwhelming the physiological tolerances of organisms, but rather by altering activity patterns or biotic interactions (Cahill et al. 2012). A study of 48 Mexican lizard species of the genus *Sceloporus* found that 12% of local populations have gone extinct from 1975–2009, with extinctions correlated with the rate of change in maximum air temperatures (Sinervo et al. 2010). Furthermore, due to the necessity of using thermal refuges, hours of restriction in activity during reproduction was higher at extinct than persistent sites, limiting time available for reproduction and compromising population growth and persistence. Additionally, as described above, changes in species' phenologies can create temporal mismatches in population explosions of predator and prey species, leading to local population extinctions. For example, in the case of two local populations of the checkerspot butterfly *Euphydryas editha bayensis* in central California, extinction was linked to an increase in extremes in annual precipitation, leading to a mismatch in plant biomass peaks and larval emergence, resulting in increased larval mortality and ultimately compromising the persistence of some local populations (McLaughlin et al. 2002). These studies support the idea that contemporary climate change has already led to local population extinctions, and is likely to continue to do so in the future as the planet continues to warm at an accelerated rate.

1.2. The potential for adaptation to climate change

1.2.1. Rapid evolution in response to environmental change

In addition to the spatial and temporal effects of climate change on biodiversity, one of the foremost questions in contemporary climate change research has been “Will species' adaptability be sufficient to allow them to avoid the negative consequences of climate change?”

A recent meta-analysis reported that local climatic influences, particularly precipitation and potential evapotranspiration, predict global variation in selection in terrestrial plant and animal populations, suggesting that global climatic shifts are already driving selection (Siepielski et al. 2017). Additionally, a growing body of evidence suggests that rapid adaptive evolution may be possible, particularly in response to anthropogenic disturbance (Reznick & Ghalambor 2001; Gingerich 2009). A key component of this body of evidence comes from observations of rapid adaptation in invasive species upon entering a non-native environment (Hendry et al. 2008; Whitney & Gabler 2008). However, studies also point to anthropogenic climate change as a driver of selection in natural populations (Reusch & Wood 2007). Some studies provide direct evidence of evolution by showing changes in climate-related phenotypic traits over time that are both genetically-based and heritable. Studies of this kind have often found that traits relating to the seasonal timing of life history events are undergoing a micro-evolutionary response to contemporary climate change, with little evidence for adjustments in the physiological optima or thermal tolerances of organisms (Bradshaw & Holzapfel 2006). For example, the longer growing seasons resulting from global warming have been associated with a shortening of the critical photoperiod in the pitcher-plant mosquito *Wyeomyia smithii*. Furthermore, this photoperiodic response was found to be genetically based with high heritability, indicating an evolutionary response to climate change that is detectable over a period of just five years (Bradshaw & Holzapfel 2001). As another example, it was found that the earlier arrival of spring and consequent changes in food abundance triggered both a plastic and genetic response in the breeding phenology of the North American red squirrel *Tamiasciurus hudsonicus*, with breeding dates advancing by about six days per generation, or 18 days over a period of ten years (Réale et al. 2003). In birds, similar phenological responses have been observed, for example in the advancement of egg-laying dates of the European great tit *Parus major* and earlier autumn migration in the Eurasian blackcap *Sylvia atricapilla* (Nussey et al. 2005; Pulido et al. 2001). In plants, genetic change in response to the selective pressures of extreme events, such as drought and frost, have been identified from observations of climate-linked genetic variation, with phenological traits such as bud set time under selection (Mimura & Aitken 2007, 2010; Jump et al. 2008). In the few instances of micro-evolution in morphological traits as a result of climate change, natural selection has acted in favour of genotypes most capable of dispersing to areas of greater climatic suitability, evidenced for example by longer wing length in insects of recently founded populations (Thomas et al. 2001).

1.2.2. Phenotypic plasticity & its evolution

Although most studies point to the ability of populations to undergo rapid adaptive evolution, the speed of evolution is not unconstrained. Aside from the limits posed by a necessity for sufficient genetic variation in climate-related traits, the heritability of this variation, and a strong selective pressure, antagonistic relationships among traits can limit adaptive evolution (Etterson & Shaw 2001). Thus, in many cases plasticity, or phenotypic flexibility (“reversible within-individual variation”), is a more likely mechanism through which organisms may respond to contemporary climate change (Piersma & Drent 2003). In fact, phenotypically plastic responses to climate change are more commonly identified than directional selection (Gienapp et al. 2008; Canale & Henry 2010). Phenotypic plasticity, rather than directional selection, may be an especially advantageous quality given that climatic instability and an increase in extreme events have come to characterize the current period of global change (Canale & Henry 2010). Whereas a microevolutionary, directional response to selection would be more favourable in areas of predictable, continuous climate warming or drying, “multi-purpose genotypes” capable of withstanding climatic fluctuations through enhanced phenotypic plasticity may be selected for in areas experiencing an increase in climatic instability (Canale & Henry 2010). Across a diverse range of taxa, particularly in ectothermic species, variation in internal or external morphological traits has been observed in response to environmental fluctuations. For example, in both iguanas and tortoises, plasticity in body size has been observed in response to variation in rainfall (Wikelski & Thom 2000; Loehr et al. 2007). In amphibians, phenotypic flexibility has been observed in gut structure and digestive enzyme activity as a result of variation in dietary composition and food quantity (Naya et al. 2005; Sabat et al. 2005). A similar phenomenon has been observed in migratory birds as they take advantage of diverse habitat conditions (McWilliams & Karasov 2001).

While phenotypic plasticity is often separated from genotypic change, a number of recent studies provide support for a genetic basis of plasticity; that is, that genetic variation for phenotypic flexibility exists, and that this variation is heritable, making plasticity a candidate trait for selection to act upon (Crispo et al. 2010). The ability of plasticity to evolve is thus dependent on genetic variation for plasticity, that is, that genomes within a population differ in their “norm of reaction”, the profile of phenotypes a single genotype is able to produce in different environments (Newman 1988; Scheiner 1993). This has been demonstrated both in the lab, by the artificial creation of a selective pressure (Driessen et al. 2007), and in the natural environment. For example, it was found that heritable genetic variation for plasticity in egg-

laying date confers an advantage to females, allowing the successful recruitment of more offspring than those females without laying date flexibility (Nussey et al. 2005). Genetic variation for phenotypic plasticity has also been observed in amphibians. For example, a study of desert-living toads in an area of variable pond duration identified the existence of plasticity in development time, which differed between sibships (Newman 1988, 1989). This supports a genetic basis for development time as well as the heritability of such variation, suggesting that plasticity in rate of metamorphosis is a trait that can evolve in response to environmental selection pressures. Importantly, microevolution and adaptive, heritable plasticity can help species to respond in the face of rapid climate change, but both are dependent on a pool of sufficiently variable genotypes to provide the raw material for these potentially rescuing processes to proceed at a rate sufficient to offset the negative effects of climate change.

1.3. Genetic footprints of contemporary and historical climate change

1.3.1. The importance of genetic diversity

Intraspecific genetic diversity is the most fundamental unit of biodiversity (May 1994), and is a major determinant of a species' evolutionary potential and resistance to stress (Hughes & Stachowicz 2004; Frankham 2005; Nowak et al. 2007). Standing genetic variation, together with newly acquired mutations, is the raw material on which natural selection acts, allowing species to adapt to environmental changes (Barrett & Schluter 2008). The increasing recognition of the importance of intraspecific genetic diversity, particularly in the context of global climate change, has led to an increasing emphasis on the preservation of within-species diversity and evolutionary processes in the conservation literature (Mace et al. 2003; Mace & Purvis 2008; Sgrò et al. 2011).

1.3.2. Contemporary climate change & intraspecific genetic diversity

Though much of the current body of climate change research has focused on the spatial and phenological shifts associated with climate change, as well as the impact of climate change at the species level, relatively few studies have addressed the potential impact of climate change on intraspecific genetic diversity (Pauls et al. 2013). Moreover, the work that has been done in this area has focused on crop species and animals of commercial importance. This lack of information on the potential effect of climate change on intraspecific diversity is especially worrying given that studies at the morpho-species level tend to drastically underestimate biodiversity loss under climate change (Bálint et al. 2011).

Given the fact that current geographical patterns of variation in intraspecific genetic diversity are driven by historical and contemporary variation in population size and gene flow, levels of genetic diversity can vary across a species' range (Vucetich & Waite 2003; Eckert et al. 2008). Due to the potential of climate shifts to modify species' ranges (Chen et al. 2011), climate change can affect the distribution of genetic variants across the landscape and can potentially cause a loss of intraspecific genetic diversity, especially when climate shifts lead to range contraction (Leblois et al. 2006; Arenas et al. 2012). A number of authors have explored the impact of climate-induced range shifts on intraspecific genetic diversity for a range of taxa, using a combination of climatic data, species distribution models, and information on variation in intraspecific diversity across a landscape. For example, in a study looking at the loss of intraspecific genetic diversity in relation to loss of climatically suitable space in nine species of aquatic insects in Europe (as revealed by species distribution modelling), it was found that an average of 84% and 68% of haplotypes could lose all of their climatically suitable space under severe and moderate emissions scenarios, respectively (Bálint et al. 2011). Another study on an endangered montane mayfly in central Europe looked at the relationship between the number of alleles and heterozygosity and patterns of habitat loss expected with climate change, finding that regional extinction is likely to cause a loss of genetic diversity, and thus a loss of adaptive potential (Taubmann et al. 2011). Using a landscape genetics approach, Tolley et al. (2009) provide additional support for the relationship between climate-induced range loss and reduction of intraspecific diversity, identifying a positive relationship between nucleotide diversity and loss of climatically suitable space in two lizards of the Cape Floristic Region of South Africa. That is, those areas expected to experience the greatest losses in climatically suitable space were those that harboured the highest genetic diversity. Taken together, these studies strongly suggest that the accelerated climatic changes we currently face could cause a loss of intraspecific diversity and negatively affect the ability of populations to respond to further environmental change. Importantly, studies such as these, which combine species distribution modelling with genetic data, can provide valuable insight that can be used to prioritise populations for conservation efforts, as they allow allocation of resources to conservation units that both harbour high levels of standing genetic variation and are expected to remain within climatically suitable space in the future (Taubmann et al. 2011).

1.3.3. The role of historical climate changes in genetic divergence

While future climate changes are predicted to cause distributional shifts in many endemic amphibians, potentially impacting patterns of genetic diversity, historical climate fluctuations

have also been implicated in clade divergence in a number of vertebrate taxa (Dynesius & Jansson 2000; Hewitt 2000). Although some argue that contemporary climate is the main driver of patterns of species diversity (Hawkins et al. 2003), many studies suggest that contemporary climate change is actually not as predictive of current patterns of species richness as historical climate, particularly in species with small ranges (Jetz et al. 2004; Araújo et al. 2008). This is important because it may mechanistically link geographical susceptibility to climate change to variation in genetic diversity.

The Quaternary Period, from about 2.6 million years ago to present day, is characterized by cyclical glacial-interglacial climatic fluctuations (Zachos et al. 2001), a phenomenon that is hypothesised to have had a marked effect on the geographical distributions of many taxa. Speciation and divergence in a number of phylogenetic groups has been traced back to this period (Avice et al. 1998; Hewitt 2000; Carstens & Knowles 2007). Two of the primary mechanisms used to explain the influence of climate on speciation, and/or diversification within species, are niche divergence and niche conservatism. Niche divergence, often called ecological or divergent speciation, occurs when different climatic conditions impose differential selection pressures on two populations of the same species, driving divergent selection in the different climatic zones (Rundle & Nosil 2005). This mechanism is often associated with climatic instability in periods of climatic oscillation, such as the Quaternary (Hua & Wiens 2013), and would predict that those areas experiencing the greatest climatic dynamism in the past would be highest in genetic diversity. Niche conservatism, on the other hand, involves microevolution in traits unrelated to climate, in areas of historical climatic stability. In this case, climate-induced distributional fragmentation can lead to the establishment of refugial populations, where climate has remained stable, and which harbour both the ancestral form and divergent forms (Peterson et al. 1999; Wiens 2004). This hypothesis would predict that areas that have experienced the greatest climatic dynamism in the past would be lowest in genetic diversity, as it is stability that allows divergence. It is important to note, that, although these processes can result in the formation of new species, they can also drive divergence without resulting in speciation.

1.4. Amphibian responses to climate change

1.4.1. The global amphibian crisis

Amphibians are among the most vulnerable taxa to climate change, with 11–15% of species both considered highly climate change vulnerable and currently carrying a threatened status

according to the IUCN Red List (Foden et al. 2013). Though amphibians have survived the past four mass extinction events in Earth's history, the imminent "sixth mass extinction" of the Anthropocene may well overwhelm their ability to keep pace with global change (Wake & Vredenburg 2008). Moreover, climate change is but one of a multitude of factors that are causing amphibian population declines, including habitat loss and degradation, disease, pollution, and non-native species. Because of these threats, amphibian populations worldwide have experienced massive declines in the past century. The Global Amphibian Assessment reported that 32.5% of about 6,300 species are threatened globally, and 43% of species have declining populations (Stuart et al. 2004). For 22.5%, the equivalent of over 1,000 amphibian species, insufficient data exists to make an assessment of threat status, meaning the actual number of threatened species is likely much higher than the reported value (Stuart et al. 2004). In fact, one author estimated that current rates of amphibian extinction are 211 times higher than natural, background extinction rates (McCallum 2007), with another study estimating that an additional 7% of all currently recognized frog species will go extinct by the end of the century (Alroy 2015).

1.4.2. Climate change and amphibian declines

Changes in climate have been correlated with a number of amphibian population declines around the world. For example, in Italy, declines of 14 species were associated with one or more climatic variables (D'Amen & Bombi 2009). In the USA's Yellowstone National Park, many amphibian populations have disappeared due to changes in hydrology (McMenamin et al. 2008). The region has been experiencing a decrease in annual precipitation and higher temperatures during the warmest months of the year, causing the number of permanently dry ponds to increase four-fold over a period of 16 years. This has resulted in the loss of more than half the amphibian populations from the northern section of the park over the same period. This finding is particularly alarming because the habitat is otherwise pristine. In South Carolina, changes in hydroperiod due to a drying trend and insufficient rainfall have been correlated with the declines of four amphibian species along the Savannah River, as fewer aquatic breeding sites are available for larval development (Daszak et al. 2005).

1.4.3. Phenological and physiological effects of climate change in amphibians

While climate change can lead directly to individual mortality and population declines, it also affects species by influencing various aspects of a species' biology, including reproduction, development, or general physiology (Blaustein et al. 2010). A growing body of evidence

suggests that climate change impacts the success of reproduction in some amphibian species (Blaustein et al. 2001). This is because temperature is a primary control on the timing of emergence and, subsequently, timing of breeding in a number of temperate species (Duellman & Trueb 1986). Additionally, the breeding peaks of permanent pond breeders are linked to temperature, while rainfall pulses are important for breeding success in temporary pond breeders (Richter-Boix et al. 2006). Consequently, climatic changes have already been linked to changes in breeding phenology. A meta-analysis of 14 long-term amphibian studies of 44 populations of 31 species showed that 28 populations were breeding earlier, by 2-60 days (Li et al. 2013). Another analysis of breeding dates of 203 species from various taxa revealed that amphibians have experienced the greatest shifts toward earlier breeding than any other taxonomic group (Parmesan 2007). Earlier breeding dates have been observed for amphibians in both temperate and tropical regions. In Ithaca, New York, four local amphibian species have started giving breeding calls about 2 weeks earlier than in the early 1900s, coinciding with an increase in mean maximum daily temperatures of 1.0-2.3°C in six months of the year between 1900 and 1999 (Gibbs & Breisch 2001). Five of the six months where temperatures increases were observed are critical for these local amphibians, since they are when overwintering, emergence, courtship, and spawning take place. A Japanese study demonstrated that date of first spawning shows a strong correlation with mean monthly temperature immediately prior to the breeding season (Kusano & Inoue 2008), and in all populations of three amphibian species examined, the authors recorded significantly earlier breeding dates over a 12-31 year period. Earlier breeding is not inconsequential for amphibian populations, as earlier emergence from overwintering has the potential to be lethal to embryos and larvae.

While the impacts of climate change on reproduction and development are immediately apparent, physiological changes linked to changes in climate are subtler, but nevertheless important in the persistence of local populations, and potentially the species as a whole. Because amphibians are ectothermic, they have no internal regulation of body temperature, and thus their metabolic rates increase as the temperature of the external environment increases, making them especially sensitive to climatic change. Some authors estimated that if the climate warms by 1.1 to 6.4°C by 2100, the metabolic rates of Southeast Asian amphibians and reptiles will increase by 10–75% (Bickford et al. 2010). This means that in order to maintain their body size, amphibians will have to eat considerably more prey items. The issue of increased metabolic rate becomes exacerbated by changes in prey availability with climate change. Macroinvertebrates, one of the main prey items of stream amphibians, are expected to decrease

in abundance with climate change, with declines of 21% for every 1°C temperature increase predicted in temperate ecosystems of the United Kingdom (Durance & Ormerod 2007). Although behavioural adaptations can help to ameliorate the detrimental effects of changing climate on metabolism, they represent trade-offs. In amphibians, behavioural adaptations to reduced water availability, increased temperature, and high levels of UV radiation include burrowing, use of refugia, and reductions in activity (Rohr & Palmer 2013). However, foraging activity decreases under dry conditions, suggesting that water conservation behaviours interfere with foraging, resulting in smaller body size and even mortality (Rohr & Palmer 2013). Therefore, through its influence on metabolism, changing climate can cause individual mortality and, potentially, the extinction of local populations in areas of high exposure, high vulnerability, and low adaptive capacity (Foden et al. 2013).

1.5. Climate change & the Cape flora and fauna

1.5.1. Historical climate shifts and speciation

Glacial-interglacial cycles associated with Milankovitch cycles are cited as a common cause for observed geographical patterns in species diversity (Dynesius & Jansson 2000). In the Western Cape of South Africa, the expansion of Antarctic sea ice during glacial periods caused a change in the direction of the westerly winds, displacing them towards the equator. This increased the proportion of winter rainfall during glacial periods and creating an arid climate in the summer months (Chase & Meadows 2007). Accordingly, the Last Glacial Maximum saw the expansion of the winter rainfall zone in the Western Cape (Cockcroft et al. 1987). The aridification of the region has also been attributed, in part, to substantial geological changes in the area, specifically the upliftment of the eastern part of the subcontinent (McCarthy & Rubidge 2005). These climatic transitions have been associated with the diversification of floral lineages that were pre-adapted to survive the increasingly arid summers, with pockets of moist climate acting as refugia for a number of species that survived this transition. Acting together, these events have been put forward as contributing factors in the generation of diversity in the Cape flora (Verboom et al. 2009).

The potential for historical climatic transitions to influence gene flow and clade divergence has been explored quite extensively in the South African flora and fauna, particularly in species of the Western Cape. In fact, historical climate fluctuations have been associated with the generation of the vast diversity of floral species in the Cape Floristic Region (CFR), which is known as a global biodiversity hotspot (Cowling et al. 1996; Myers et al. 2000; Richardson et

al. 2001). Associated with the endemic fynbos biome of the region is a diverse endemic faunal assemblage, with diversification in an array of groups attributed to historical climate changes (Daniels et al. 2006; Price et al. 2007). A number of studies have highlighted the impact of past climatic fluctuations, during the Miocene, Pliocene, or Pleistocene, on divergence in the herpetofauna of the Cape (Matthee & Flemming 2002; Daniels et al. 2004; Tolley et al. 2006, 2008). Studies of the region's endemic amphibians, in particular, have emphasized the importance of past climatic transitions in driving range disjunction and/or cladogenesis, including studies of the endemic Cape platanna *Xenopus gilli*, and the western leopard toad *Sclerophrys pantherina* (Measey & Tolley 2011; Fogell et al. 2013). This suggests that even relatively recent climate changes, in the past 1,000-5,000 years, may be associated with genetic divergence within species. These inferences from genetic work are corroborated by findings from climate modelling studies, which suggest that, in support of the hypothesis originally proposed by herpetologist John Poynton in 1964 (Poynton 1964), the current distribution of the Cape amphibian fauna is reflective of historical climatic fluctuations, and that the current distributions of the Cape frogs represent refugial areas where climate was more stable (Schreiner et al. 2013).

1.5.2. Future climate projections for South Africa

A general trend of drying in the west and an increase in precipitation in the east is predicted for South Africa (Tadross et al. 2005; Hewitson & Crane 2006). Climate projections for the country, derived from multiple global climate models, project a decrease in winter rainfall in the Western Cape of 10-40% by 2070-2100 (Engelbrecht et al. 2009), with annual rainfall in the southwestern Cape projected to decrease by about 20%, leading to a significant reduction in mean annual runoff by 2050 (Meadows 2006). Because climatic influences have been shown to be important in clade divergence in a number of Cape lineages, it is not surprising that contemporary climate change is projected to influence range dynamics in Western Cape taxa. Although there is always a degree of uncertainty in climate change projections, these climatic changes, in conjunction with ongoing land transformation, are predicted to have negative consequences for the flora of the region. For example, Midgley et al. (2002, 2003) predict a loss of 51 to 65% of the fynbos biome area by 2050, depending on future greenhouse gas concentrations, with many species of the iconic Proteaceae projected to experience range contractions or eliminations.

1.6. Cape amphibian endemism & threat

Contemporary climate change, which threatens the persistence of the fynbos biome as a whole, is also predicted to have a significant impact on the Cape's fauna, particularly amphibians. The amphibians of South Africa provide an important opportunity to study the impact of climate change on genetic diversity, as many are endemic, threatened, and generally considered to be poor dispersers, limiting their ability to respond to projected climatic changes through rapid range shifts. Of the 118 described amphibian species in the country, 51 species (43%) are endemic (Measey 2011). Unfortunately, this high level of endemism is accompanied by a high degree of threat. Two of the primary threats to important breeding areas of terrestrial and stream amphibians in South Africa are land transformation (for agriculture, energy production, mining, and residential and commercial development) and alien invasive species (Measey 2011; Mokhatla et al. 2012). These threats are likely to be exacerbated by increasingly rapid climatic shifts in the southwestern part of the country, which have the potential to impact survival and recruitment in this moisture-dependent group.

Because the onset of winter rainfall coincides with the breeding seasons of many amphibians in the southwestern part of the country (Minter et al. 2004), a decrease or delay in winter rainfall could impact breeding success and/or recruitment and could lead to loss of climatically suitable area. Between the periods 1905–1995 and 1996–2003, 70% of South African frog species have likely experienced range contractions, attributed mainly to land-use change and climatic shifts, with many experiencing upslope shifts of an average of nearly 50 meters (Botts et al. 2015). Furthermore, the small range size and narrow habitat and climate niches of many CFR amphibians makes them particularly vulnerable to range contractions due to contemporary climate change (Botts et al. 2013). Projections of future distributions of the CFR amphibians using species distribution modelling suggest that species' range loss will be accelerated under future emissions scenarios, particularly for amphibians of the Western Cape (Mokhatla et al. 2015). In a study of 37 of the 40 species endemic to the CFR, it was found that the amphibian assemblage has already lost 56% of climatically suitable space since the Last Glacial Maximum (LGM), 21,000 years ago. Furthermore, the study predicted a north-eastward shift of the CFR assemblage at a greater rate than has been observed during past periods of climatic change, with landscape fragmentation increasing under future climate scenarios, affecting the ranges of lowland species particularly severely (Mokhatla et al. 2015). Given these predictions of range contraction and fragmentation, an exploration of their effects on intraspecific genetic diversity is warranted, particularly for those species that are currently facing multiple threats.

1.7. Study species

The western leopard toad *Sclerophrys pantherina* is a coastal fynbos species with a disjunct distribution from the Cape Peninsula in the west to Pearly Beach in the east (Minter et al. 2004). *S. pantherina* generally occupies lowland habitats, and breeds in permanent water bodies and some temporary wetlands from July to September, with peak breeding in August cued by high winter rainfall. The species is classified on the IUCN Red List as Endangered, and is threatened mainly by habitat loss and fragmentation associated with urban development (IUCN SA-FroG 2016; Measey 2011; de Villiers 2004). Due to its urban distribution, the species is frequently killed on the roads while moving between fragmented breeding sites. Though the species is relatively tolerant of habitat alteration, and can forage and breed in transformed landscapes such as urban gardens and agricultural lands, the pace of habitat transformation continues to cause a decline in numbers, with subpopulations in Kleinmond, Betty's Bay, and Pringle Bay now considered extinct (Measey 2011).

A previous genetic study investigated whether the disjunct distribution of *S. pantherina* may be anthropogenically driven. Using mitochondrial DNA analyses, the authors found that gene flow between the two disjunct populations in the Cape Metropole and Overstrand, separated by 100 km, ceased about 1,200 years ago, and likely coincided with a range contraction resulting from a drying period in the Holocene (Measey & Tolley 2011). This suggests that intraspecific divergence may have been driven by these relatively recent climatic shifts, and that the species may be sensitive to future climate change.

1.8. Aims & objectives

Much climate change research to date has focused on impacts on higher-level biodiversity, such as populations, species, communities, or biomes. However, the potential effects of climatic shifts on the most fundamental level of biodiversity, genetic variation within populations or species, have been largely understudied. A full understanding of the genetic consequences of climate change is required if we are to assess the long-term effects of climate change on biodiversity. In this project, I aim first to explore the potential impact of future climatic shifts on the genetic landscape of *S. pantherina*. Second, I aim to explore the possibility of a mechanistic link between climatic instability and genetic diversity, which may explain the potential overlap of high diversity areas with areas of high climatic fluctuation.

I had two hypotheses regarding the relationship between climate and genetic diversity. My first hypothesis was that if areas that experienced high climatic instability in the past are epicentres

of genetic divergence (i.e. climate-driven niche divergence), and also correspond to areas of high future climatic change, then areas projected to experience the greatest losses in climatically suitable space will overlap with areas of high genetic diversity. This hypothesis would have severe implications from a conservation perspective. My second hypothesis was that genetic divergence is driven by climatic stability and divergence in refugial populations (i.e. climate-driven niche conservatism). If past climatic stability is predictive of future climatic stability, then areas that have experienced stable climate in the past, and are projected to experience stable climate in the future, will overlap with areas of high clade diversity. This hypothesis would have less severe implications from a conservation perspective because it would imply the greatest losses of climatically suitable space in areas of relatively low diversity. I tested these hypotheses by combining historical and future range projections from species distribution models with information on spatial patterns of intraspecific diversity in *S. pantherina*.

My objectives were as follows:

- 1) To characterize levels of intraspecific genetic diversity across the geographical distribution of *S. pantherina*
- 2) To project the potential distribution of *S. pantherina* based on climate projections for 2050 and 2070, as well as the mid-Holocene (~6,000 years before present)
- 3) To determine if climatic stability through time (mid-Holocene-present) is associated with high genetic diversity, and if areas that are predicted to become climatically unsuitable in the future overlap with areas of high genetic diversity
- 4) To determine if local past climatic stability is predictive of local future climatic stability

Overlaying spatial genetic structure and climate models can provide a preliminary assessment of the potential impact of climate change on genetic landscapes and gene flow, and aid our ability to predict species' vulnerability in the face of future environmental change. At a practical level, identifying areas of high genetic value and high risk of range loss and fragmentation due to climate change can contribute to the prioritization of specific populations (evolutionary significant units) for conservation action. At a broader scale, the results of this study could provide insight into our understanding of the general relationship between historical and future projected climatic fluctuations and genetic divergence.

2. Methods

2.1. Tissue sampling & laboratory work

The majority of the tissue samples used in this study were gleaned from the herpetological tissue bank at SANBI. Additional tissue samples were collected by toe clipping, using road surveys and directed searches around known breeding areas to locate individuals during the peak of the breeding period, in late August. Some samples were also taken from road-killed toads contributed by volunteer groups. This resulted in coverage of eight sample sites in the Cape Metropole and five sample sites in the Overstrand Municipality (Figure 2.1). Newly collected tissue samples were stored in salt-saturated 20% dimethyl sulphide (DMSO) to maintain the integrity of the DNA for laboratory work, while samples from the SANBI tissue bank were stored in ethanol. Small pieces of tissue (approximately 25 mg) were excised from the toe clippings for DNA extraction. Prior to DNA extraction, samples that were stored in DMSO were soaked three times in double-distilled water for a period of one hour to remove any excess salt which would interfere with amplification. The tissues were then dried in a vacuum centrifuge to remove excess volatiles. Total genomic DNA was extracted from the tissue samples using MacManes salt extraction protocol (MacManes 2013).

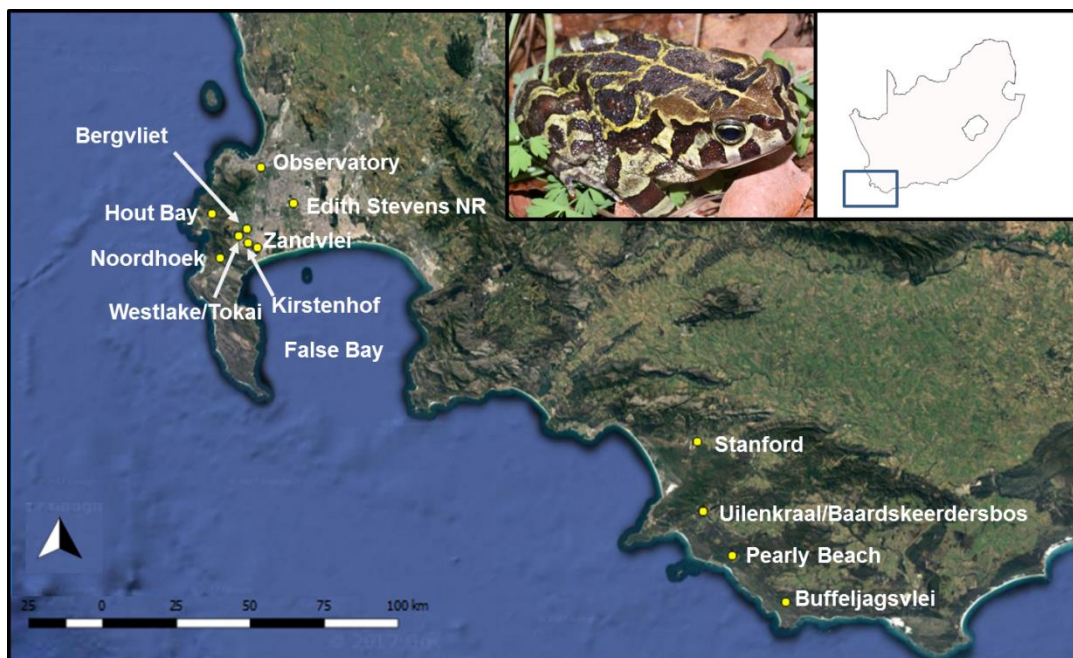


Figure 2.1. Map of the southwestern corner of the Western Cape, South Africa showing sample sites of *Sclerophrys pantherina* represented in this study.

Amplification of a portion of the mitochondrial NADH dehydrogenase 2 (ND2) gene was carried out by the polymerase chain reaction (PCR) using the toad-specific primer set vMet2

& vTrp (Cunningham & Cherry 2004). PCR was performed in 25 μ l reaction volumes using the reagents and quantities given in Table 2.1 in the MultiGene OptiMax Thermal Cycler (Labnet International Inc.). PCR conditions consisted of an initial denaturation of 4 minutes at 95°C followed by 30 cycles of denaturation (94°C, 30s), annealing (55–58°C, 45s), and extension (72°C, 1 min), terminating with a final extension of 10 minutes at 72°C. PCR products were quantified by electrophoresis on a 0.8% agarose gel with ethidium bromide and visualized under UV light. Successfully amplified products were sent to Macrogen Inc. (Amsterdam, Netherlands) for Sanger sequencing using the forward primer vMet2.

Table 2.1. Quantity of reagents used in PCR amplification of the ND2 mitochondrial region. Purified double distilled water was added to a total volume of 25 μ l per PCR reaction.

Reagent (concentration)	Volume (μ l)
Taq polymerase (5u/ μ l)	0.15
MgCl ₂ (25mM)	2.5
Buffer	2.5
DNTPs	0.4
F Primer (105 ng/ μ l)	0.3
R Primer (105 ng/ μ l)	0.3
DNA (30-70 ng/ μ l)	2.0

2.2. Phylogenetic analyses

Phylogenetic analyses were carried out to ensure that all individuals sampled had been correctly identified, and to examine the clustering of individuals from localities across the species' range. Sequence data from the previous population genetic study of *S. pantherina* (Measey & Tolley 2011) were combined with the newly generated sequences to obtain a representative sample from across the species' distribution, yielding a combined sequence dataset of 222 individuals from the 13 sample sites (Table 2.2). In addition, the ND2 sequences of two individuals of *Sclerophrys rangeri* were derived from GenBank, and one individual of *Sclerophrys garmani* was sequenced for use as outgroup taxa in constructing the phylogeny (Cunningham & Cherry 2004). Sequences were aligned by progressive pairwise alignment in Geneious 8.1.7 (Kearse et al. 2012) using default parameters. A maximum likelihood (ML) approach was used in phylogenetic reconstruction, using 741 base pairs of the ND2 marker. The best-fitting model

of nucleotide substitution was determined according to the Akaike Information Criterion (AIC) test using the programme jModelTest (Posada 2008). The test selected a simple Jukes-Cantor (JC) model as the best-fit model. This model assumes equal frequencies of the nucleotide bases and equal substitution rates across all nucleotides. A maximum likelihood analysis was performed using the programme RAxML with 1000 bootstrap repetitions (Stamatakis 2014).

Table 2.2. Total number of ND2 mitochondrial DNA sequences of *Sclerophrys pantherina* from each sample site in the Overstrand and Cape Metropole that were used in analyses. Sequences were compiled from previous work (Measey & Tolley 2011) and generated in this study.

Locality	n
Stanford	8
Baardskeerdersbos	24
Uilenkraal	12
Buffeljagsvlei	8
Pearly Beach	28
Overstrand (total; this study)	80; 38
Zandvlei/Lakeside	13
Tokai/Westlake	7
Kirstenhof	18
Bergvliet/Constantia	27
Hout Bay	12
Noordhoek	35
Observatory/Pinelands	18
Edith Stevens Nature Reserve	12
Cape Metropole (total; this study)	142; 34

2.3. Population genetic analyses

To increase statistical power of the analyses, sites with low sample size (<5 individuals), were combined with the nearest sampling site (yielding the 13 sample sites shown in Table 2.2). To detect fine-scale genetic structure, several methods were used. First, to visualize the relationships of sequences between sample sites, a median-joining network (Bandelt et al.

1999) was created using the software PopArt 1.6 (<http://popart.otago.ac.nz>). Representing genetic relationships within a species is best done using a network approach, such as median-joining, as low divergence, polytomy, and persistent ancestral nodes often characterise intraspecific relationships, making it difficult to represent gene evolution using a traditional bifurcating tree (Posada & Crandall 2001).

A spatial analysis of molecular variance (SAMOVA) was used to determine the level of differentiation between sampling sites (Dupanloup et al. 2002). This analysis uses spatial information for the sample sites, and requires that subjective groups be created *a priori*. SAMOVA was run with K=2-10 groups in order to determine the grouping that maximizes the value of FCT, the proportion of the total variation due to differences between groups. A maximum K value of 10 was deemed sufficient, as previous population genetic analyses suggest minimal population structure (Measey & Tolley 2011). A gamma value of 0.0 was used in SAMOVA, corresponding to the gamma distribution of the best-fit model determined in the phylogenetic analyses. Haplotype and nucleotide diversity were also estimated for each of the groups defined by SAMOVA using the Arlequin population genetics software version 3.5 (Excoffier & Lischer 2010).

2.4. Genetic landscapes

Spatial patterns of genetic diversity were represented by 3D landscapes created in Alleles in Space version 1.0 (Miller 2005). In this analysis, a spatial network is constructed which connects all sampling locations using Delaunay triangulation. This triangulation method satisfies the condition that no vortex must lie within the circumscribing circle of any triangle in the triangulation (Brouns et al. 2003). Genetic distances between sampling locations are then computed at the midpoint between two sampling points, and genetic distances are interpolated across the landscape surface (Miller 2005). In the resulting 3D plot, genetic distances are represented by surface heights. This analysis was used because it allows for the examination of inter-individual genetic distances across the geographical distribution of a species, rather than providing a single measure of genetic distance between pre-determined “populations.” Due to the discontinuous nature of the species’ distribution, separate genetic landscapes were constructed for the Cape Metropole and the Overstrand. If a single landscape were to be generated, any interpolated points between the two regions would be an artefact of genetic differences between the two areas, and would not be based on any actual underlying samples

in this area, where the species does not occur. For ease of interpretation, these 3D landscapes were translated into 2D space using Quantum GIS (QGIS 2016).

2.5. Species distribution modelling

Species distribution modelling (SDM) has become a popular tool that is implemented in a number of conservation contexts, mainly in conservation planning and projecting species' distributions into future climates. In conservation planning, SDMs are used to design protected area networks (Peterson & Kluza 2003; Williams et al. 2005), to identify areas of potential human-wildlife conflict (Brotons et al. 2004), and to locate areas of high conservation value for endangered species (Wilson et al. 2011). In the context of climate change, SDMs are frequently used to determine how species' ranges and patterns of habitat suitability across a landscape will change under future climate scenarios (Midgley et al. 2003; Thomas et al. 2004). In the case of the latter, two main types of approaches are used: process-based and correlative (Dormann et al. 2012). In the process-based approach, known functional traits of a species and its physiological tolerances are used to predict its occurrence across a landscape. In the correlative approach, a species' occurrence is predicted based on the assumption that the current distribution is in equilibrium with the environment, allowing the species' bioclimatic associations to be determined from points of known occurrence (Pearson & Dawson 2003). Although the latter approach has flaws (Davis et al. 1998; Dormann et al. 2012), it is widely used to provide a preliminary assessment of the impact of climate change on species' distributions, and it is used in this manner in the present study.

2.5.1. Occurrence data

Occurrence data for *S. pantherina* and background data were obtained from a dataset of South African frog species records. This dataset was compiled from multiple sources, and included records from the South African Frog Atlas Project (SAFAP; Minter et al 2004), iSpot, VertNet, Cape Nature, Ezemvelo Kwa-Zulu Natal Wildlife, Mpumalanga Tourism and Parks Agency, the South African Institute for Aquatic Biodiversity, the Endangered Wildlife Trust, museums, and personal observations made by members of the DNA lab at SANBI and others. A total of 3,244 records of *S. pantherina* were obtained from this dataset. These data were cleaned in R v3.3.2 using the package *biogeo*, and all subsequent data manipulation and modelling steps were carried out using this platform (R Core Development Team 2016). Data cleaning involved identifying records that were plotted in the sea and moving them to the nearest cell on land. Additionally, any records without both longitude and latitude coordinates were removed from

the dataset prior to running the model. This resulted in a cleaned dataset of 3,211 records, all of which were used in building a sampling bias model in order to inform the model about relative sampling effort across the species' distribution. When building the SDM incorporating the bioclimatic predictors and spatial priors, the occurrence records were aggregated to a resolution of 30 arc seconds, removing duplicate records within grid cells so that the resolution of occurrence data matched that of the climate data. This resulted in a dataset of 142 records of *S. pantherina*, all of which were used as occurrence points to inform the SDMs. This was done under the assumption that both breeding sites and foraging area limit the distribution of the species.

Additionally, a background dataset was compiled to inform the model about the full range of environmental variables in the study region. Here, background data were chosen to represent a “target group,” a group of species for which occurrence records have been collected in a similar manner or using similar equipment, often of the same broad taxonomic group (Ponder et al. 2001). The assumption is that, because the records were collected in a similar manner, a similar sampling bias would be exhibited in both the background and presence datasets, minimizing sampling bias as a confounding factor in the projected distributions. Choosing background samples in this way, as opposed to choosing background samples from the study region randomly, has been shown to improve model performance across a range of modelling methods (Phillips et al. 2009). Accordingly, the background dataset was comprised of all occurrence records in the data set for which a full scientific name was available and which fell within the model extent. Background data were also cleaned according to the procedure described above, yielding 23,174 records which were used in building the sampling bias model. As was done with the occurrence data for *S. pantherina*, background data were aggregated to a resolution of 30 arc seconds, removing duplicates in grid cells. This resulted in the retention of 16,357 records to be used in building the SDM.

2.5.2. Climate data

All climate data used in modelling were obtained from WorldClim v1.4 (Hijmans et al. 2005). Selections of the 19 bioclimatic variables at a resolution of 30 arc seconds were used to represent the current climate in the study area. To project future distributions, down-scaled future climate projections for 2050 (average for 2041–2060) and 2070 (average for 2061–2080) were obtained from three general circulation models (GCMs) used in the Fifth Assessment of the IPCC (Table 2.3). The average of the predictions from the three GCMs for each variable

was used in building the model in order to account for variability between model projections. SDMs were projected for each future time period based on two IPCC greenhouse gas scenarios, or Representative Concentration Pathways (RCPs): RCP 2.6, corresponding to a mean 1°C warming (“best-case scenario”), and RCP 8.5, corresponding to a mean 2.0°C warming within the specified time period (“worst-case scenario”). In addition, to test the hypothesis that historical climatic stability drives genetic diversification, species distributions were projected onto paleoclimate models from the mid-Holocene, approximately 6,000 years ago (Table 2.3). These historic climate data were at a resolution of 30 arc seconds. As in projecting the future distributions, the average of the outputs of multiple GCMs for each variable was used as an input to the mid-Holocene model (Table 2.3).

Table 2.3. General circulation models from which climate data were derived for modelling the distribution of *Sclerophrys pantherina* in future (2050 & 2070) and past (mid-Holocene, 6,000 years before present).

Time Period(s)	Model Name	Citation
Future; mid-Holocene	Hadley Centre Global Environment Model 2- Earth System (HADGEM-ES)	Collins et al 2011
Future; mid-Holocene	Meteorological Research Institute-Coupled Global Climate Model 3 (MRI-CGCM3)	Yukimoto et al 2012
Future; mid Holocene	Community Climate System-4 (CCSM4)	Gent et al 2011

2.5.3 Model setup & evaluation

A maximum entropy approach (Maxent) was used to model the species’ distribution in current, future, and past time periods (Phillips et al. 2006a). This approach, as with other correlative modelling methods, uses the environmental variables associated with points of known occurrence to predict the climatically suitable habitat of the species across a geographical area, assuming that the current distribution is the best indicator of the habitat requirements of the species (Pearson & Dawson 2003). A species’ distribution can then be projected onto future or past climate environments. The Maxent approach uses a background dataset, showing the full spectrum of environmental conditions in the study area, as a basis for comparison with the set of environmental conditions presence sites, using these datasets to predict a relative probability of observing the species in each grid cell of the study area (Elith et al. 2011). Maxent has been shown to perform generally well compared to models which rely only on presence data, without

including background data, and is especially useful in predicting species distributions from small sample sizes (Elith et al. 2006; Hernandez et al. 2006).

Here, the Maxent model was constructed and implemented in R v3.3.2, using an approach described by Merow et al. (2016), which provides a useful extension on the Maxent framework. Whereas a typical Maxent model assumes uniform priors in geographical space, that is, that there is an equal probability of detecting a species anywhere on the landscape, the minimum entropy approach (Minxent) described by these authors utilizes spatially explicit prior information informing the model on the relative probability of a presence being recorded across the study region, where the spatial projection of the model is constrained by these spatial priors. These spatial priors can be supplied in the form of “nuisance offsets,” variables that could confound predictions. Here, gridded data on distance to roads and population density (available from CIESIN 2013, 2005, respectively) were incorporated as a nuisance offset. This was done under the assumptions that areas near roads and with high population densities would be sampled disproportionately to their availability, a likely reality due to the urban distribution of the species and the high probability of detecting toads on roads with heavy traffic, particularly during the breeding season. These data layers, along with the target group presence records were used to run an initial sampling bias model, the output of which was then used as a nuisance offset in the Minxent model incorporating the environmental predictors characterizing the distribution of *S. pantherina*. The model was projected to the extent of the fynbos biome, as *S. pantherina* is a fynbos endemic species and is therefore unlikely to find suitable habitat conditions outside of this vegetation type.

Inter-correlation among bioclimatic variables was assessed by calculating Pearson’s correlation coefficient. Because correlative SDMs can be sensitive to variable selection, particularly when spurious correlations between species’ occurrence and environmental predictors exist, multiple combinations of uncorrelated ($r > 0.75$), ecologically relevant bioclimatic variables were used to build the Minxent model, in order to investigate the models’ dependence on variable selection. Selecting variables based on their ecological relevance has been shown to maximize model transferability across space (Petitpierre et al. 2016), and thus is likely also to improve model projections into novel climate environments.

The ecologically relevant bioclimatic variables used in building the SDMs were variables describing annual and daily temperature variation, as well as levels of precipitation in the coldest, wettest time of the year, which are ecologically relevant when considering the biology

of the species, and have been found to be critical in determining the distribution of other amphibian species using both correlative SDM and mapping of physiological tolerances. First, it has been found that temperature range and extremes, rather than average temperature, drives the distribution of critical thermal maxima in ectotherms (Clusella-Trullas et al. 2011). In particular, mean diurnal temperature range, and other derived temperature variation metrics (i.e. isothermality) which use mean diurnal range in their calculation, have been found to be important contributors to climate suitability models of amphibian species around the world, including SDMs of the amphibian community of the Cape Floristic Region (Keith et al. 2014; Urbina-Cardona 2010; Zank et al. 2014; Mokhatla et al. 2015). Second, diurnal temperature range is an important driver of physiological tolerance, with one study identifying this variable as the single best predictor of critical thermal maximum in ectotherms (Clusella-Trullas et al. 2011). Third, because *S. pantherina* breeds from the end of July to the beginning of September, the amount of rainfall occurring during this period, usually the coldest quarter of the year, is critical for the persistence of the species. Indeed, this variable has been found to be important in contributing to habitat suitability models for a variety of CFR amphibians, including *S. pantherina* (Mokhatla et al. 2015).

Each modelled scenario was run 50 times, and the averaged output of all model runs was used to determine the relative importance of each of the selected variables, and was presented as the final prediction for each scenario. The relative occurrence rate (ROR) for each cell of the gridded environmental data was presented, as presence-only data precludes the prediction of absolute occurrence rates because population size is unknown (Fithian & Hastie 2012). The ROR describes the probability that a cell contains a presence site relative to other cells (Merow et al. 2013), and is essentially a measure of climatic suitability (the two terms are used here interchangeably). The model fit was evaluated using a jack-knife approach (Merow et al 2016). Overall model performance was evaluated using the area under the receiver operating characteristic (AUC). This is the most commonly used statistic to evaluate SDMs, and in the context of presence-only approaches such as Maxent, it measures the probability that the model gives a higher score to a random presence site than to a randomly selected site from the study area (Phillips et al. 2009). However, because the reliability of AUC as a model evaluation measure has been criticized, due in part to its sensitivity to the models' spatial extent (Lobo et al. 2007), three additional model evaluation statistics were reported: model sensitivity (the proportion of presences predicted correctly), specificity (the proportion of absences predicted

correctly), and the True Skill Statistic (TSS) (a prevalence-independent metric; Allouche et al. 2006).

Additionally, a jack-knife resampling test was used to determine the importance of individual variables in building the model. This test works by running the model multiple times, excluding one variable each time, and measuring the resulting reduction in model fit, as measured by AUC, regularized training gain, and test gain. Regularized training and test gain measure how much better the modelled distribution fits the training or test data, respectively, than a uniform distribution (Gormley et al. 2011). The model is then created with each variable on its own, and model fit is evaluated in the same way.

2.6. Testing the relationship between genetic diversity and climatic stability

A generalized additive model (GAM) was used to test the hypothesis that climatic stability drives genetic diversification, versus the alternative hypothesis that climatic instability drives genetic diversification. Interpolated genetic distance (as described in section 2.4) was used as the response variable, and the change in ROR (i.e. suitability) between the mid-Holocene and present was used as the predictor variable. These values were calculated by extracting the value of the difference between the mid-Holocene and present ROR at each of the points where an interpolated genetic distance was calculated.

Because separate genetic landscapes were generated for the Cape Metropole and the Overstrand (to avoid including the unsampled area between the two regions where the species does not occur) the interpolated genetic distances for the two regions were then combined into a single point dataset, from which climatic suitability values were then extracted for analyses. This was so that a potential relationship between genetic diversity and changes in climatic suitability through time could be explored looking across the whole distribution. “Region” (i.e. Cape Metropole or Overstrand) was incorporated in the models as a fixed effect, because this variable is likely to explain some variance in observed diversity, particularly because inter-individual diversity in Overstrand was likely to be higher, because the sites are further apart. Expectedly, the inclusion of “region” resulted in a lower AIC (i.e. better model fit) and thus was retained in all models using the interpolated genetic distances.

Five “knots” were used to delimit the predictor variable, allowing polynomial functions to be fit to each of the four resulting sections separately. A GAM allows for a smoother model fit, and does not impose a linear relationship on the data. The AIC for the GAM fitted in this way

was lower than that of the equivalent linear model by more than 2 units, and thus it was selected over the linear model. To determine whether geographical areas that are expected to lose climatically suitable space in the future overlap with areas of high genetic diversity, we examined the relationship between the interpolated genetic distances and the change in ROR between future and present, using a GAM fitted in the same manner described above.

To eliminate the uncertainty and non-independence involved in relating two modelled variables (interpolated genetic distance and ROR), the relationship between the change in ROR between future and present and the actual haplotype and nucleotide diversity values at each of the 13 sample sites was also examined. Assuming that the surrounding climatic conditions, not just the point location where samples were collected, would influence a sample site, the average change in ROR in a buffer area of 0.05 decimal degrees (equivalent to about 6 km) around each sample site was used in calculating the changes in suitability between time periods, rather than the specific value at the point of sample collection. Here, separate linear regressions were used to determine whether the change in ROR between the mid-Holocene and present, or the change in ROR between future and present, were predictive of nucleotide or haplotype diversity at the sample sites. A linear model was chosen because a GAM fitted with the same variables did not result in a lower AIC, so the simplest model was selected.

A linear regression was also used to determine whether local past climatic stability is predictive of local future climatic stability, with the change in ROR between the mid-Holocene and present (at each point where an interpolated genetic distance was calculated) used as the predictor variable, and the change in ROR between present and future time periods at each interpolated genetic distance points used as the response variable. Here, a positive relationship between these two variables would indicate that historical stability is predictive of future stability in an area. All of the above analyses were performed on the projections of both Model A and Model B, for all future time periods and RCPs.

Although the above methods are useful in addressing the hypotheses that climatic stability or instability drive diversification, to address the practical question of where losses in climatically suitable space are predicted to be most extreme, it is helpful to look at changes in climatic suitability between the Cape Metropole and the Overstrand. Therefore, I extracted the changes in suitability between future and present at each of the interpolated genetic distance points and plotting these against longitude, allowing me to quantitatively examine

changes in suitability between the two regions, which are shown qualitatively by the suitability maps generated by the SDMs.

3. Results

3.1. Phylogenetic analyses

The maximum likelihood tree confirmed that all samples were correctly identified as *S. pantherina*, as there were no outlying branches. In general, samples from the Overstrand clustered together and samples from the Cape Metropole clustered together, indicating that they are genetically distinct (Figure A.1, Appendix A).

3.2. Population genetic analyses

The SAMOVA showed that the sequence dataset is optimally divided into K=2 groups, as this maximizes the value of the among group variation (FCT) (Figure 3.1). These two groups conclusively corresponded to the populations of the Cape Metropole and Overstrand. The SAMOVA also revealed that most of the genetic variance (59%) was explained by differences among groups, with only a small proportion of the variation (<6%) explained by differences among specific sample sites within these two larger groups (Table 3.1). The high value of FCT relative to FSC (variance between sample sites) supports the conclusion that variation between sample sites is low compared to the variation between the Cape Metropole and Overstrand groups.

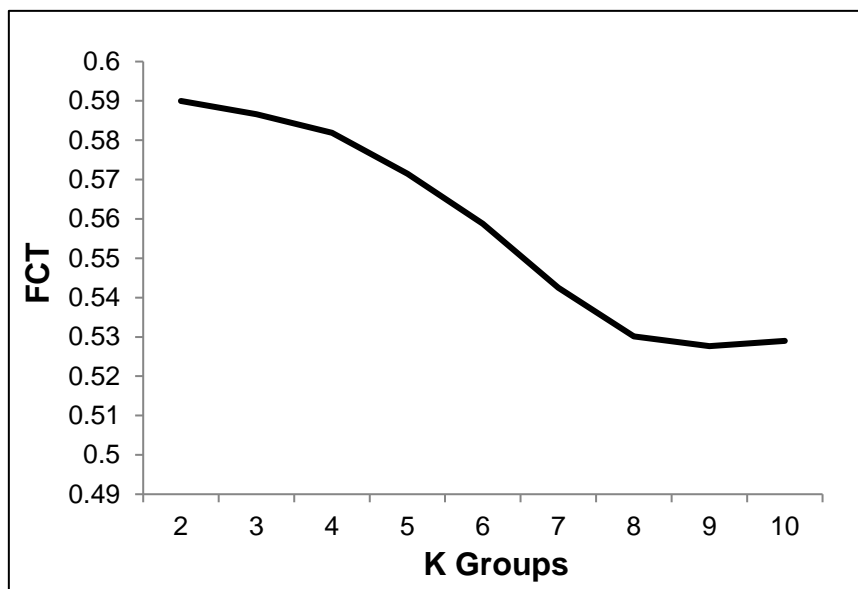


Figure 3.1. Proportion of total genetic variation in mitochondrial ND2 sequences of *Sclerophrys pantherina* due to differences between groups of sequences (FCT), for varying numbers of groups. Variance between groups is maximized at K=2 groups.

Table 3.1. Results of a spatial analysis of molecular variance (SAMOVA) performed on mitochondrial ND2 sequences of *Sclerophrys pantherina*, with associated fixation indices quantifying levels of genetic differentiation at different hierarchical scales. FSC= variance between populations (sample sites) within groups, FST= variance between sample sites relative to the total sample, FCT= variance between groups.

Source of variation	df	Sum of squares	Variance	% of variation
Among groups	1	72.607	0.69229	59.00
Among populations within groups	11	16.084	0.06396	5.45
Within populations	209	87.187	0.41716	35.55
Total	221	175.878	1.17342	
Fixation Indices				
FSC: 0.13295				
FST: 0.64449				
FCT: 0.58998				

A total of 19 haplotypes were identified in the sequence dataset, with 12 haplotypes represented in the sample sites of the Cape Metropole and 9 represented in the sample sites of the Overstrand (Table 3.2). The haplotype network supports the grouping identified by SAMOVA, showing that the two groups have unique haplotype compositions, with only two haplotypes in common between the two (Figure 3.2). The populations of the Cape Metropole had higher nucleotide and haplotype diversity than the populations of the Overstrand (Table 3.2; Figure 3.3).

Table 3.2. Nucleotide (π) and haplotype (h) diversity in mitochondrial ND2 sequences compared between the populations of *Sclerophrys pantherina* in the Cape Metropole and Overstrand. Standard deviation (SD) for the sampling process is shown.

Area	No. of haplotypes	π (SD)	h (SD)
Cape Metropole	12	0.0014 (0.0010)	0.7153 (0.0318)
Overstrand	9	0.0010 (0.0009)	0.6070 (0.0483)

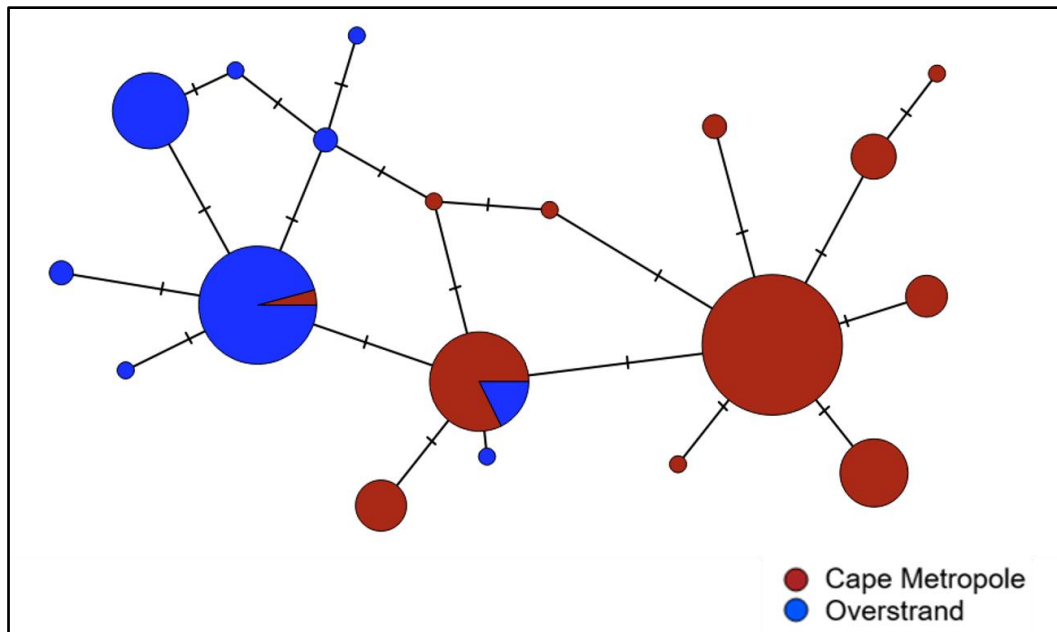


Figure 3.2. Median-joining haplotype network showing mitochondrial ND2 haplotypes of *Sclerophrys pantherina*. Tick marks indicate single nucleotide polymorphisms, while circle size is proportional to the number of individuals of each haplotype. Colour coding shows the origin of individuals possessing each haplotype.

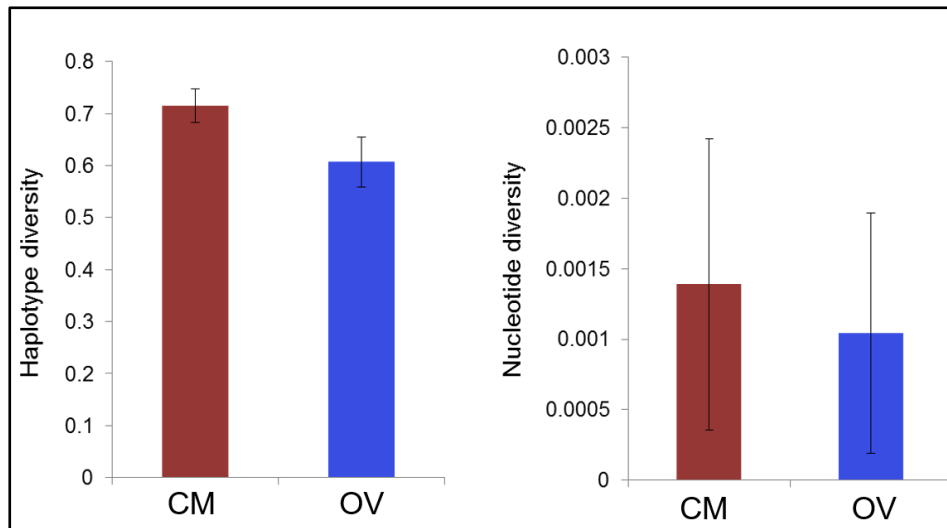


Figure 3.3. Haplotype and nucleotide diversity in mitochondrial ND2 sequences compared between the populations of *Sclerophrys pantherina* of the Cape Metropole (CM) and Overstrand (OV). Error bars represent the standard deviation for the sampling process used to compute the two values.

As indicated by the SAMOVA, differences in haplotype diversity between sample sites within the Cape Metropole or the Overstrand were generally small. However, Hout Bay and Observatory had substantially lower haplotype diversity compared to the other Cape Metropole populations, with only two haplotypes represented in each (Figure 3.4). Non-overlapping 95% intervals (Figure B.1, Appendix B) indicated that Observatory is significantly lower in haplotype diversity than all other populations in the Cape Metropole, and that Hout Bay is significantly lower in haplotype diversity than the Tokai/Zandvlei, Noordhoek, and Kirstenhof populations. Kirstenhof and Zandvlei had the highest haplotype diversities overall, and Buffeljagsvlei had the highest diversity among the populations of the Overstrand (Figure 3.4).

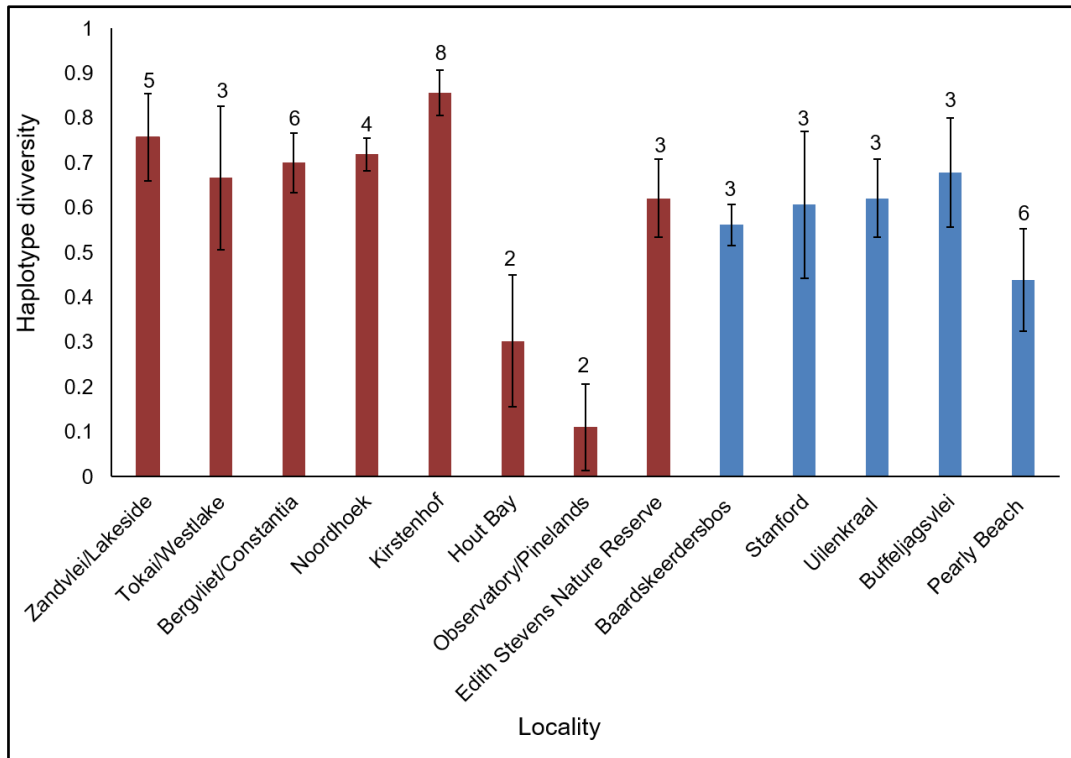


Figure 3.4. Haplotype diversity in mitochondrial ND2 sequences of *Sclerophrys pantherina* from each sampled locality. Error bars show the standard deviation of the sampling distribution. Red bars represent localities in the Cape Metropole, and blue bars represent localities in the Overstrand. Numbers above bars indicate the number of haplotypes present at each locality. Note: haplotype diversity values are calculated based on number of haplotypes and sample size, so a greater number of haplotypes does not necessarily equate to higher haplotype diversity relative to another site with fewer haplotypes; sample size per site are reported in Table 2.2.

3.3. Genetic landscape analyses

3D landscapes showed a background of relatively low diversity, with concentrated, high diversity peaks both in the Cape Metropole and Overstrand (Figure 3.5a; 3.6a). The 2D representations indicate that there are some localities harbouring particularly high and low diversity in both regions (Figure 3.5b; 3.6b). Of the localities of the Cape Metropole, the greater Zandvlei area has relatively high diversity, along with the Noordhoek area, while Observatory showed low levels of diversity, in accordance with the population genetic results. In the Overstrand, the more northern localities, Stanford and Uilenkraal, exhibited relatively low levels of diversity relative to the more southeastern localities, which were more genetically distinct from each other.

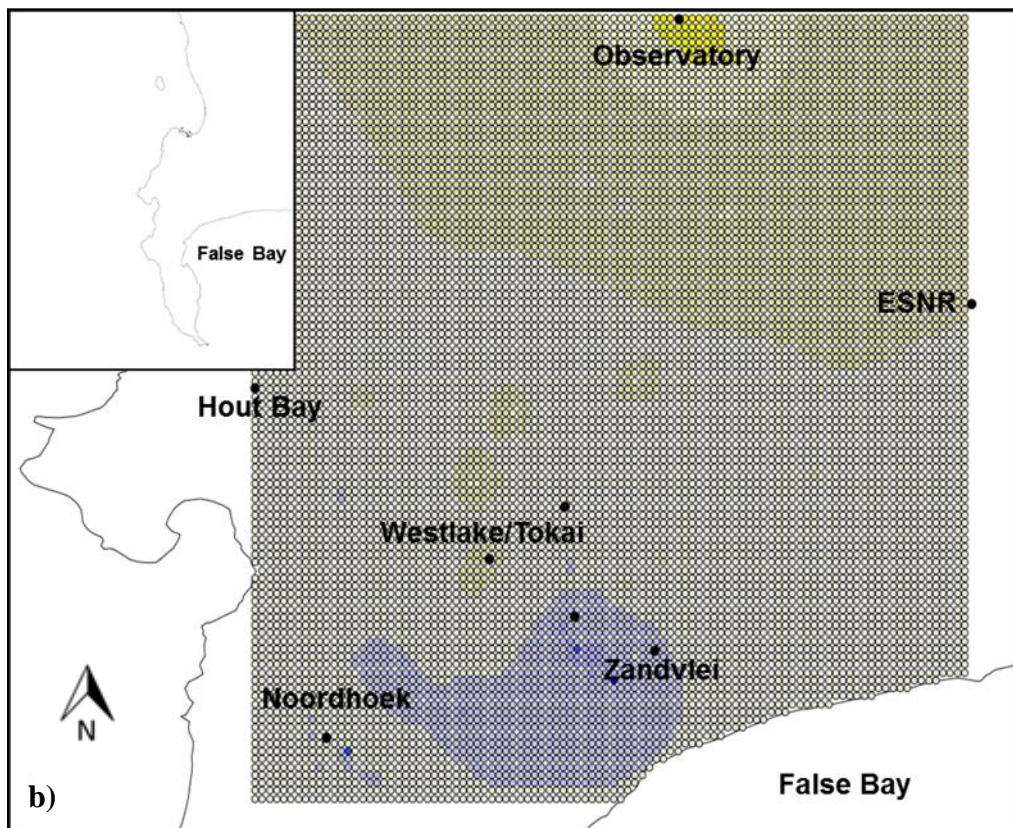
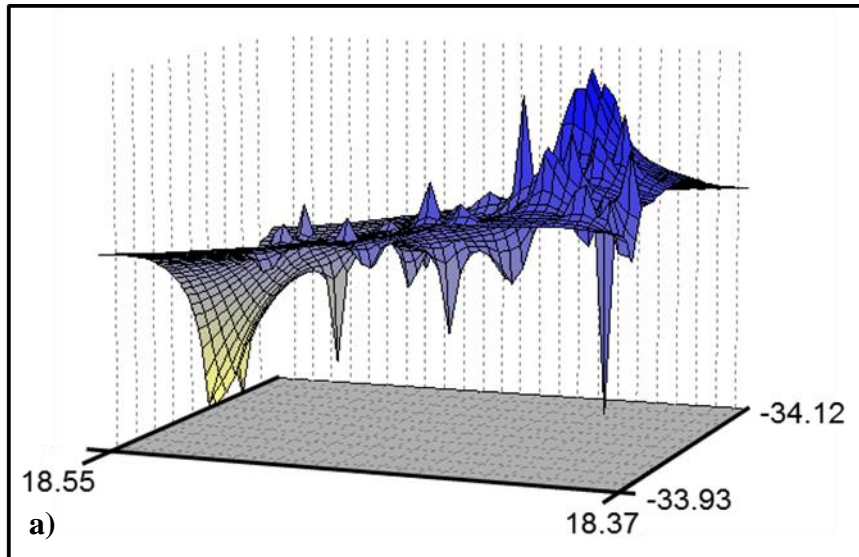


Figure 3.5 (a) 3D genetic landscape showing interpolated relative genetic distances across the distribution of *Sclerophrys pantherina* in the Cape Metropole. Surface heights correspond to levels of inter-individual diversity seen at each geographical location. (b) 2D translation of the 3D genetic landscape, with dark blues indicating areas of relatively high genetic distances between individuals, and yellows indicating areas with relatively low genetic distances. ESNR= Edith Stevens Nature Reserve.

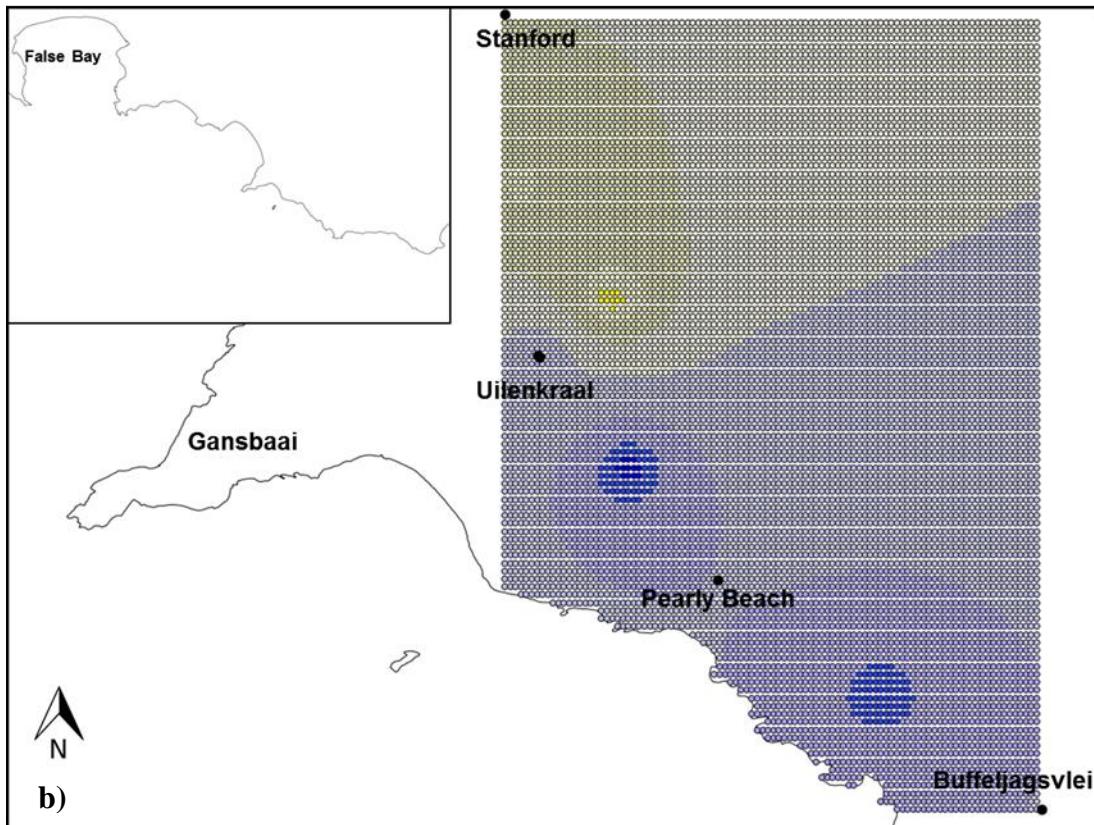
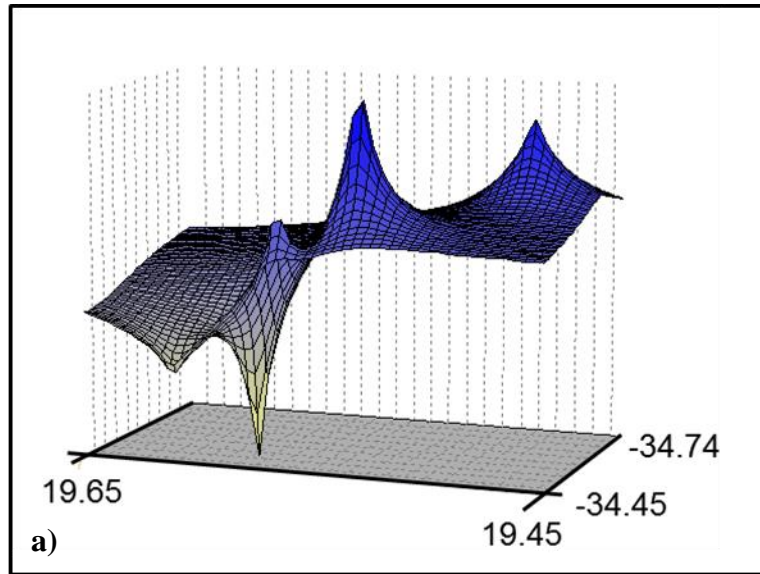


Figure 3.6. (a) 3D genetic landscape showing interpolated relative genetic distances across the distribution *Sclerophrys pantherina* in the Overstrand. Surface heights correspond to levels of inter-individual diversity seen at each geographical location. (b) 2D translation of the 3D genetic landscape, with dark blues indicating areas of relatively high genetic distances between individuals, and yellows indicating areas with relatively low genetic distances.

3.4. Species distribution modelling

There was little variation in the current species' distribution predicted by models using different combinations of bioclimatic predictors. However, variable selection had a substantial effect on the projections of the future and past distributions. To represent some of this variability, I include both the current and projected distribution of two models: first, the highest performing model that was run based on the chosen model evaluation criteria described in section 2.5.3 (Model A), and second, an additional model built using an alternative set of bioclimatic predictors presumed to be relevant based on the species' ecology (Model B). The selected model evaluation criteria indicated that both models fit the training data well (Table 3.3). The variables used in building both models are given in Tables 3.4 and 3.5. The current species' distribution predicted by both models covered the current area of occurrence, and also predicted habitat suitability in some coastal areas outside of the species' current area of occurrence (Figures 3.7; 3.8). In both models, measures of temperature variability and precipitation in the coldest or wettest time of the year were most important in predicting the current distribution of the species. The most important variables in building Model A, according to permutation importance, were mean diurnal temperature range and precipitation of the coldest quarter (Table 3.4). The jack-knife tests of regularized training gain, as well as of AUC and test gain identified precipitation of the coldest quarter, as the most important variable, on its own, in predicting the species' occurrence (Figure C.1, Appendix C). There was an overall positive relationship between precipitation in the coldest quarter and ROR, while ROR peaked at a relatively low mean diurnal temperature range (Figure 3.9a).

In Model B, temperature seasonality and precipitation in the wettest month had the highest permutation importance (Table 3.5), and the jack-knife tests of regularized training gain, test gain, and AUC identified precipitation in the wettest month as by far the most important variable, on its own, in building the model (Figure C.2, Appendix C). ROR peaked at a relatively low temperature seasonality, while there was an overall positive relationship between ROR and precipitation in the wettest month (Figure 3.9b).

Table 3.3. Performance of two Minxent models built using different environmental predictors, as measured by four model evaluation statistics: sensitivity (the proportion of presences predicted correctly), specificity (the proportion of absences predicted correctly), the area under the receiver operating characteristic (AUC), and the true skill statistic (TSS). Model A represents the best performing model considering all four metrics, and was built using ecologically relevant environmental predictors. Model B represents a model built using an alternative set of ecologically relevant environmental predictors.

Model	Sensitivity	Specificity	AUC	TSS
A	0.953	0.975	0.987	0.928
B	0.953	0.967	0.985	0.920

Table 3.4. Relative importance of variables used in Minxent **Model A** (the highest performing model), as measured by percent contribution and permutation importance.

Code	Description	% contribution	Perm. importance
BIO2	Mean diurnal temp. range	37.9	53.0
BIO19	Precipitation coldest quarter	40.4	31.8
BIO17	Precipitation driest quarter	8.0	9.3
BIO1	Annual mean temp.	13.7	5.9

Table 3.5. Relative importance of variables used in Minxent **Model B** (built from an alternative set of bioclimatic variables assumed to be relevant to the species' ecology), as measured by percent contribution and permutation importance.

Code	Description	% contribution	Perm. importance
BIO4	Temp. seasonality	26.6	39.7
BIO13	Precipitation wettest month	31.0	21.9
BIO17	Precipitation driest quarter	3.0	15.2
BIO11	Mean temp. coldest quarter	16.2	14.6
BIO15	Precipitation seasonality	23.2	8.6

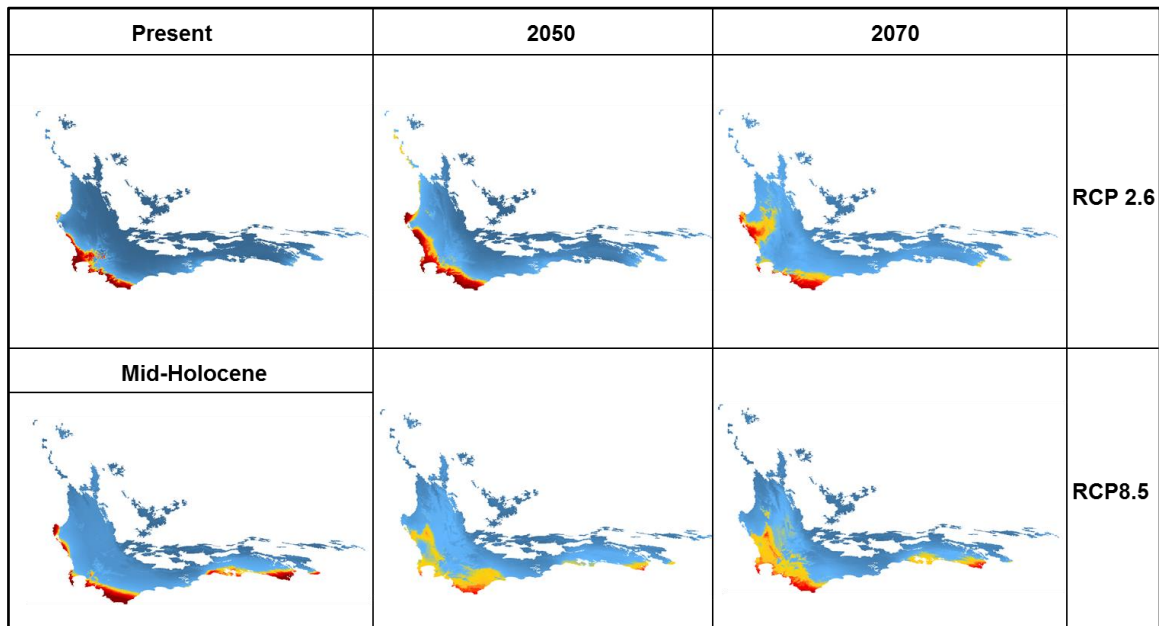


Figure 3.7. Habitat suitability maps derived from Minxent **Model A**, the highest performing model, for two future time periods under two IPCC Representative Concentration Pathways (RCPs), representing best (RCP2.6) and worst case (RCP8.5) greenhouse gas trajectories. Warmer colours indicate a higher relative occurrence rate (ROR).

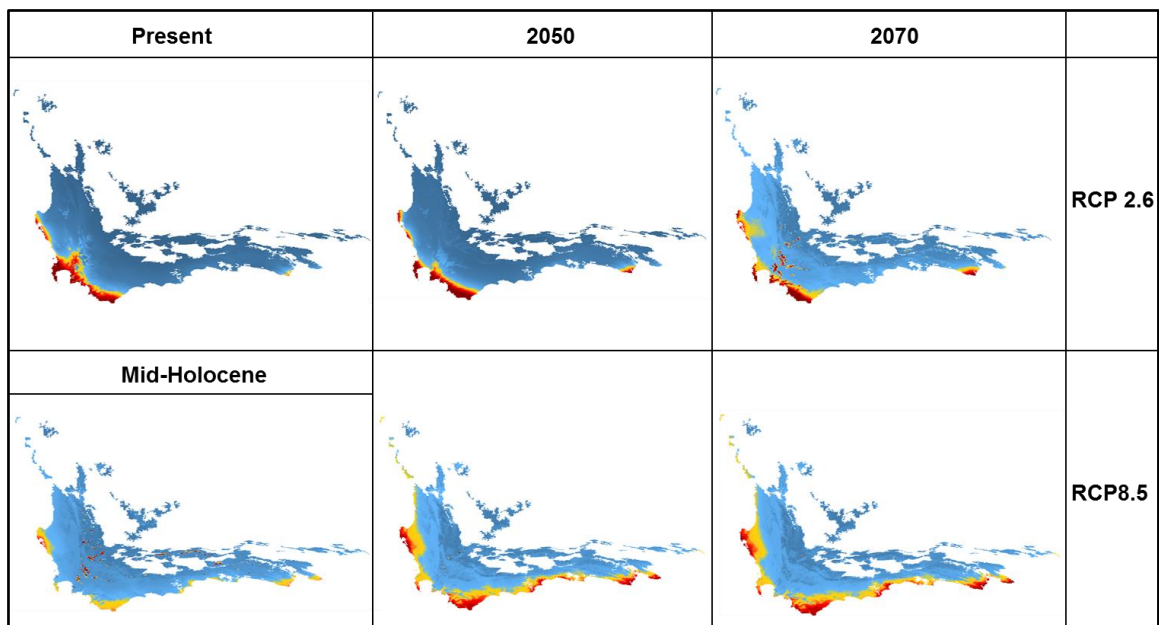


Figure 3.8. Habitat suitability maps derived from Minxent **Model B**, built from an alternative set of bioclimatic variables assumed to be relevant to the species' ecology, for two future time periods under two IPCC Representative Concentration Pathways (RCPs), representing best (RCP2.6) and worst case (RCP8.5) greenhouse gas trajectories. Warmer colours indicate a higher relative occurrence rate (ROR).

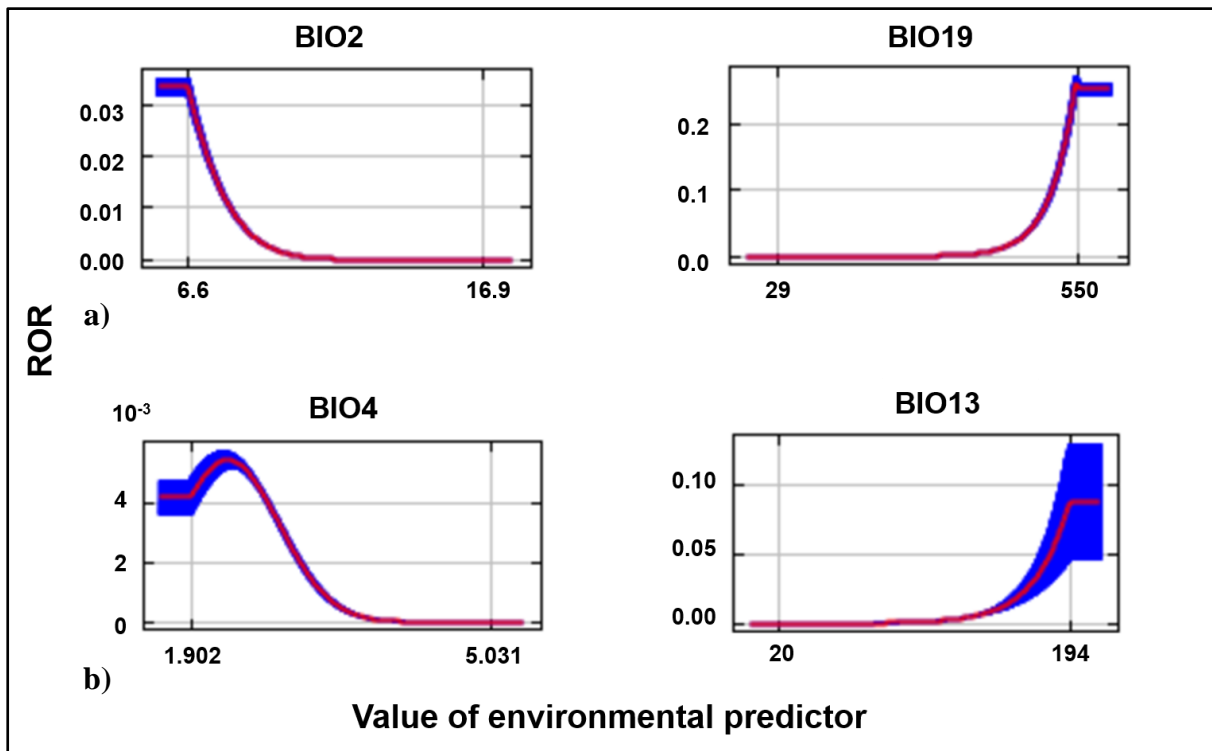


Figure 3.9. Response curves generated by Maxent, showing the relationship between the relative occurrence rate (ROR) of *S. pantherina* and the values of the most important bioclimatic variables used in building **a)** Model A: the highest performing model as indicated by model performance measures and **b)** Model B: built from an alternative set of bioclimatic variables assumed to be relevant to the species' ecology. BIO2= mean diurnal temperature range, BIO19= precipitation in the coldest quarter, BIO4= temperature seasonality, BIO13= precipitation in the wettest month.

There was considerable variation in the distributions projected by both models, associated both with the projected time period and the RCP (Figures 3.7; 3.8). Both models predict only a small decrease in climatic suitability in the species' current area of occurrence by 2050, based on RCP2.6. However, greater losses in climatically suitable space are predicted by 2070 based on this RCP, with especially severe losses projected for the current area of occurrence in the Cape Metropole (Figures 3.7; 3.8). The future projections under RCP8.5 show even greater losses in climatically suitable space in the Cape Metropole, with the Overstrand less severely affected. These losses in suitability are accompanied by gains in suitability outside the native range, particularly to the northwest of the current range, around the Langebaan area, as well as along the coastline to the east of Agulhas. Model B also predicts a fragmented distribution in the mountains inland of its current distribution. The predicted distributions for the mid-Holocene, according to both models, suggest that the species' range has not changed drastically in the

past 6,000 years, but that it has expanded inland and eastward from the Cape Peninsula towards the Cape Flats.

3.5 Testing the relationship between genetic diversity and climatic stability

Reported below are the results of analyses performed on the projections of Model A, with 2050 always used as the future time period, under RCP2.6. However, the results of all analyses for Model B and for all other modelled scenarios are given in Appendix D, and the differences between the scenarios are summarised below.

First, I carried out the GAM analysis of all interpolated genetic distance values. This revealed that change in suitability between the mid-Holocene and present was a significant predictor of interpolated genetic distance ($R^2= 0.609$; $p<0.001$; Table 3.6). Higher interpolated genetic distances were generally associated with areas of relatively high loss in suitability from the mid-Holocene to present (Figure 3.10a). Additionally, change in climatic suitability between future and present was a significant predictor of interpolated genetic distance ($R^2= 0.641$; $p<0.001$; Table 3.6). Relatively high interpolated genetic distances were associated with areas of high loss in suitability between the future and present, as well as high gains in suitability between time periods, as indicated by the “U-shape” of the curve to the left of the 0-line (Figure 3.10b). In essence, those areas expected to gain suitability with the future climatic shifts predicted for 2050 are of relatively high diversity, and those areas expected to lose the most climatically suitable space are also high in diversity. Additionally, “Region” (included in both models as a fixed effect) was also a significant predictor of interpolated genetic distances ($p<0.001$), with where there were generally high *interpolated* genetic distances identified in the Overstrand (a pattern also apparent from Figures 3.5b and 3.6b). Summarily, areas expected to experience a relatively stable future climate environment are relatively low in diversity, and those areas expected to experience a high degree of change in suitability are relatively high in diversity. Importantly, these patterns were generally robust to changes in the model, future time period, or RCP used, when looking at p-values (Table D.1; D.2, Appendix D). However, a better model fit was obtained when using the projections of Model A in analyses, as indicated by higher values of R^2 and the percentage of deviance explained by the model (Table 3.6; Table D.1, Appendix D).

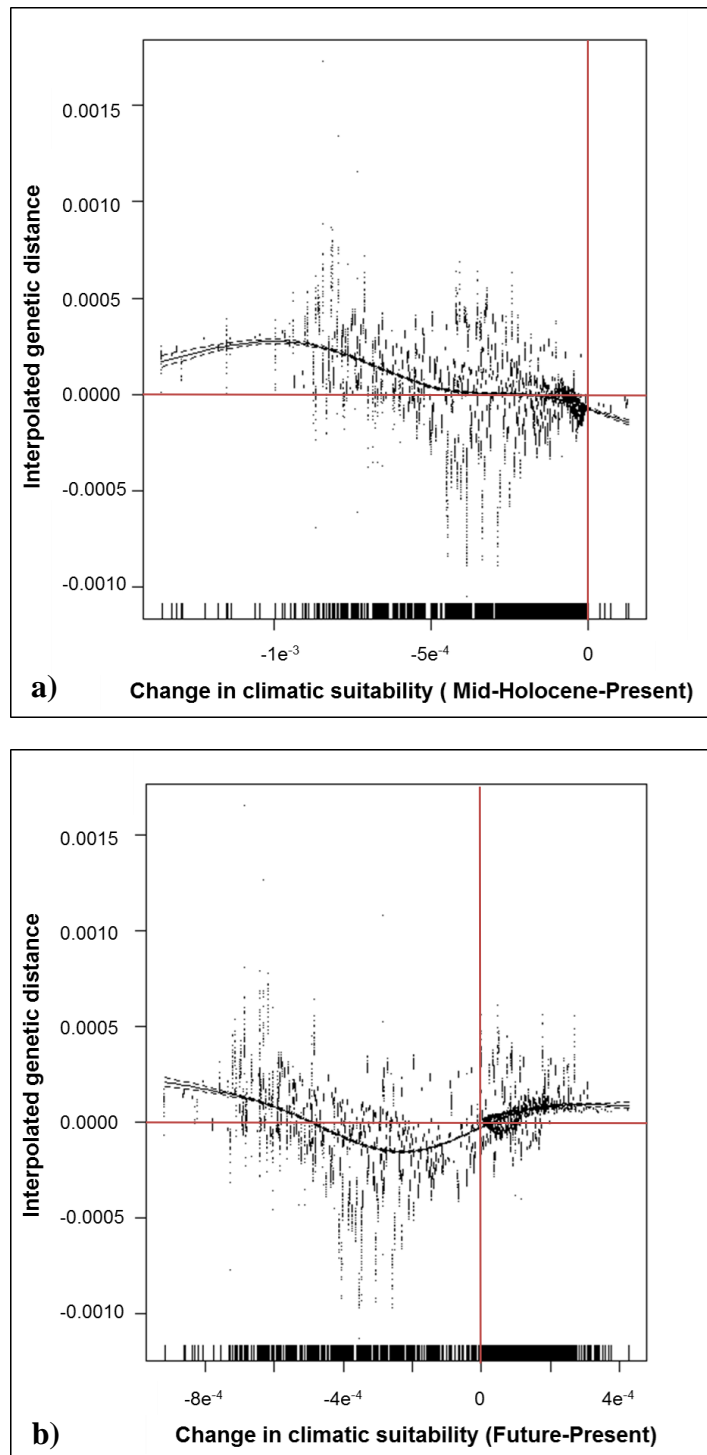


Figure 3.10. **a)** Change in climatic suitability between the Mid-Holocene and present (as projected by Model A, the highest performing Maxent model) plotted against the interpolated genetic distances at each point where a value was interpolated. **b)** Change in climatic suitability between 2050 and present, under RCP2.6 (Model A) plotted against the interpolated genetic distances. Adding in the 0-lines in these plots shows that at most genetic diversity points, there was a loss in climatic suitability between Mid-Holocene and present, and between present and future. In the case of the latter, the 0-line also indicates that areas expected to gain suitability are of intermediate diversity.

Table 3.6. Results of general additive models (GAMs), with interpolated genetic distance as the response variable, and either the difference in relative occurrence rate between Mid-Holocene and present (past climatic stability) **or** the difference in relative occurrence rate between 2050 (RCP 2.6) and present (future climatic stability) as the predictor variable. Suitability differences were derived from the projections of Model A (the highest performing Maxent model), and in both GAMs, “region” (Cape Metropole or Overstrand) was incorporated as a fixed effect and was a highly significant predictor of genetic diversity ($p < 0.001$). GAM results include the degrees of freedom (df), the F-statistic (F), p-value (p), as well as the adjusted R^2 and the percentage of deviation explained by each model (Dev. Exp. %).

Variable	df	F	p	R² (adj.)	Dev. exp. (%)
Past climatic stability	4	894.7	<0.001	0.609	60.9
Future climatic stability	4	1373	<0.001	0.641	64.1

Next, I related actual haplotype and nucleotide diversity values for each of the 13 sample sites to predicted changes in climatic suitability between time periods (as projected by Model A). Similar trends emerged: there was a general trend toward higher loss in climatically suitable space between the mid-Holocene and present (Figure 3.11) in areas of relatively high haplotype and nucleotide diversity. The relationship between nucleotide diversity and change in suitability between the mid-Holocene and present was statistically significant ($R^2=0.326$; $p=0.024$; Tables 3.7 and 3.8). Similarly, greater losses in climatically suitable space were predicted in areas of relatively high nucleotide and haplotype diversity, in the more genetically diverse populations. Looking at haplotype and nucleotide diversity, these populations were those of the Cape Metropole, while gains were predicted in the less diverse Overstrand (Figure 3.12). This reflects the change shown in the suitability maps produced by the SDMs. Although the relationship between nucleotide diversity and change in suitability between future and present was not significant when looking at the projections of Model A for 2050 based on RCP2.6, the relationship became significant (at the level of $p < 0.05$) when the projection for 2070 was used, if any projections under RCP8.5 were used, or if Model B was used (Table D.4, Appendix D). This was the only case in all analyses where a relationship changed in significance at the level of $p < 0.05$ when the RCP, time period, or model varied (Table D.3; D.4, Appendix D). However, in general when looking at the relationships between both the haplotype and nucleotide diversity values and change in suitability between future and present, using projections for RCP8.5 and for the year 2050 showed better model fit than when using projections for RCP2.6 or for the year 2070, indicated by higher coefficients, adjusted R^2 , percentage of deviance explained, and p-values when these projections were used (Table D.3;

D.4, Appendix D). Regardless of this variation in model fit, all trends were in the direction predicted by the hypothesis that climatic instability is a driver of diversification.

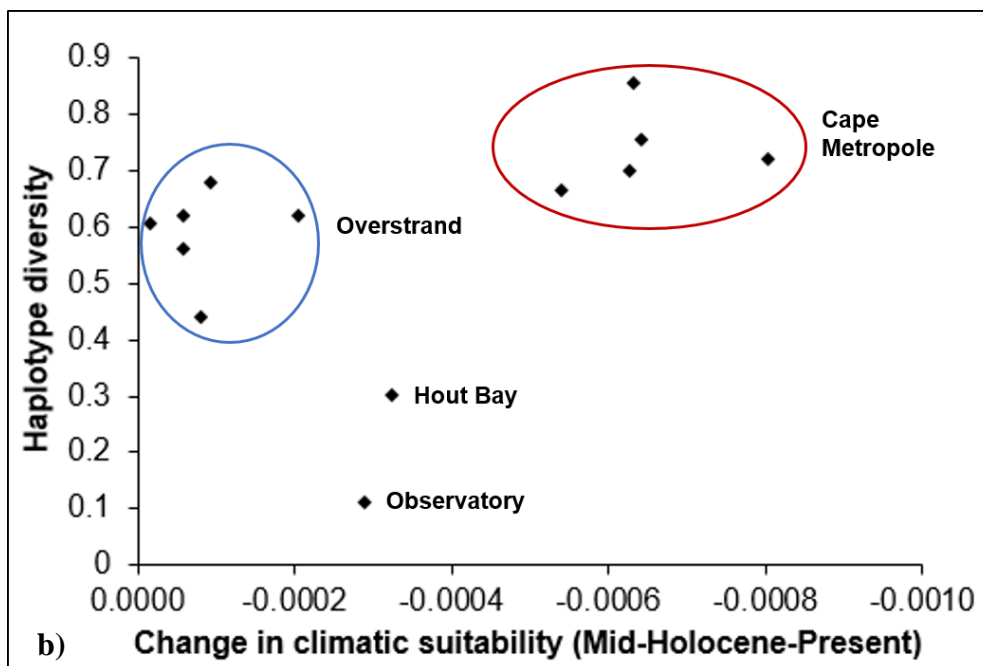
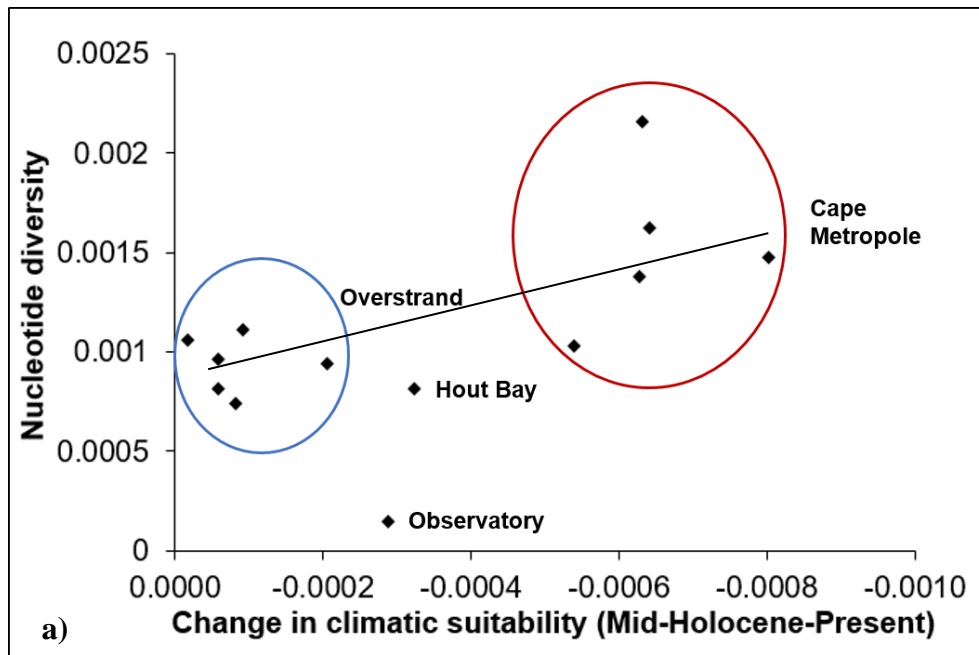


Figure 3.11. Change in relative occurrence rate (climatic suitability) between Mid-Holocene and present (from Model A, the highest performing Minxent model) plotted against **a)** nucleotide diversity for each sample site- here change in climatic stability was a significant predictor of nucleotide diversity **b)** haplotype diversity for each sample site. The circles labelled “Cape” and “Overstrand” identify the sample sites in the two areas of the distribution, with the low diversity sites at Observatory and Hout Bay lying outside these clusters. Past climatic stability was not a significant predictor of haplotype diversity in the linear regression, and did not explain a large percentage of the deviance, therefore a trendline is not shown.

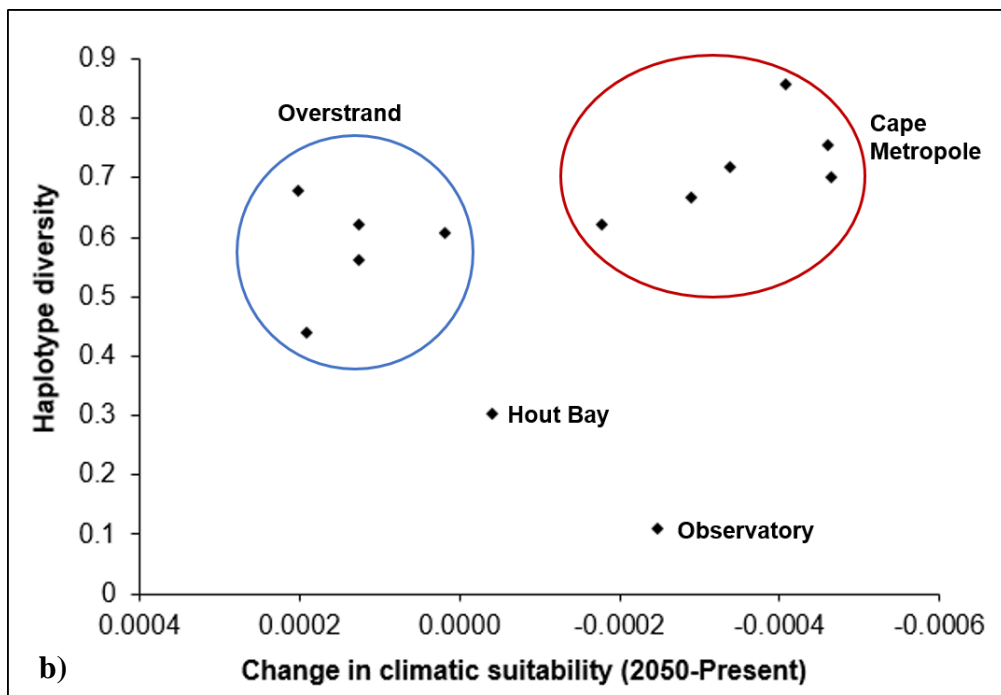
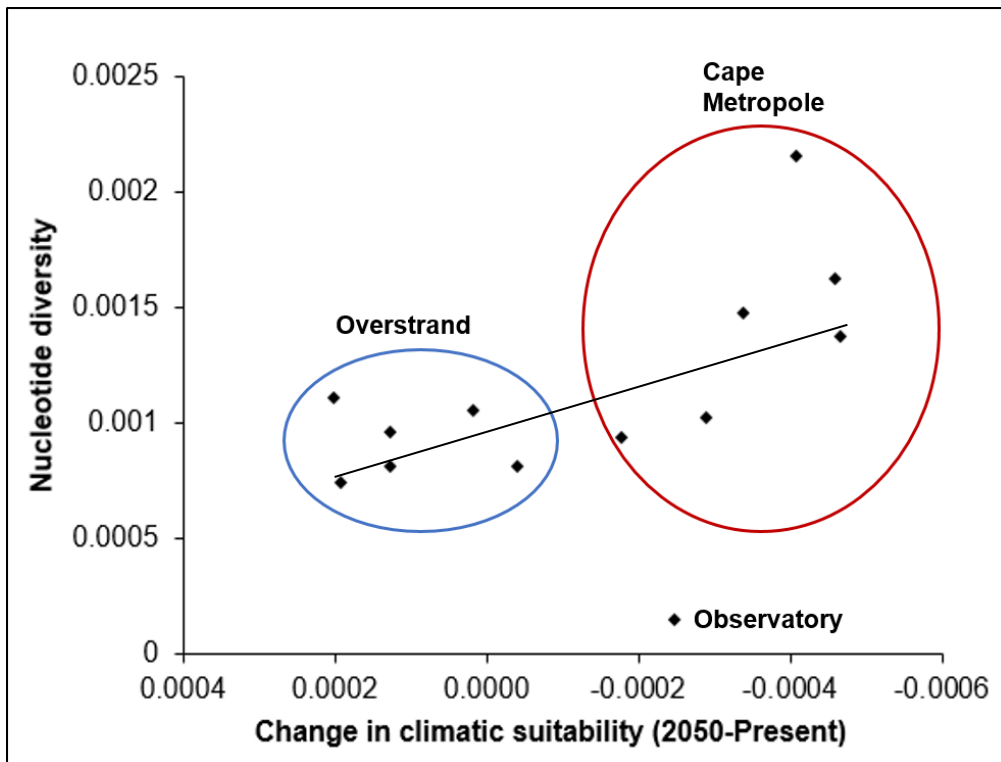


Figure 3.12. Change in relative occurrence rate (climatic suitability) between 2050 and present, under RCP2.6 (Model A, the highest performing Minxent model) plotted against **a)** nucleotide diversity for each sample site **b)** haplotype diversity for each sample site. The circles labelled “Cape” and “Overstrand” identify the sample sites in the two areas of the distribution, with the low diversity sites at Observatory (and Hout Bay in b) lying outside these clusters. Future climatic stability was not a significant predictor of haplotype diversity in the linear regression, and did not explain a large percentage of the deviance, therefore a trendline is not shown.

Table 3.7. Results of linear regressions with **haplotype** diversity values for the 13 sample sites as the response variable, and either the difference in relative occurrence rate between mid-Holocene and present (past climatic stability) **or** the difference in relative occurrence rate between 2050 (RCP 2.6) and present (future climatic stability) as the predictor variable. Both differences were derived from the projections of Model A. Regression statistics shown include the regression coefficient (Coeff) and its standard error (SE coeff), the t-value (t), p-value (p), adjusted R², and the percentage of deviation explained by each model (Dev.exp %). Degrees of freedom was equal to 11 for both models.

Variable	Coeff	SE (coeff)	t	p	R ² (adj.)	Dev. exp. (%)
Past climate stability	-293	198	-1.473	0.167	0.0899	16.6
Future climate stability	-253	227	-1.114	0.289	0.0198	10.1

Table 3.8. Results of linear regressions with **nucleotide** diversity values for the 13 sample sites as the response variable, and either the difference in relative occurrence rate between mid-Holocene and present (past climatic stability) **or** the difference in relative occurrence rate between 2050 (RCP 2.6) and present (future climatic stability) as the predictor variable. Both differences were derived from the projections of Model A. Regression statistics shown include the regression coefficient (Coeff) and its standard error (SE coeff), the t-value (t), p-value (p), adjusted R², and the percentage of deviation explained by each model (Dev.exp %). Degrees of freedom was equal to 11 for both models.

Variable	Coeff	SE (coeff)	t	p	R ² (adj.)	Dev. exp. (%)
Past climate stability	-1.087	0.417	-2.607	0.024	0.326	38.2
Future climate stability	-1.003	0.502	-1.998	0.071	0.200	26.6

Past climatic stability was found to be a significant predictor of future climatic stability in an area, as the two variables showed a strong linear relationship (Coeff= 0.911; SE coeff= 0.003484; $R^2= 0.791$; $p<0.001$; Figure 3.13). This linear relationship became even stronger ($R^2>0.900$) when projections for 2070, RCP8.5, or Model B were used, as well as when projections for each of the 13 sample sites, rather than for all the interpolated points, were used.

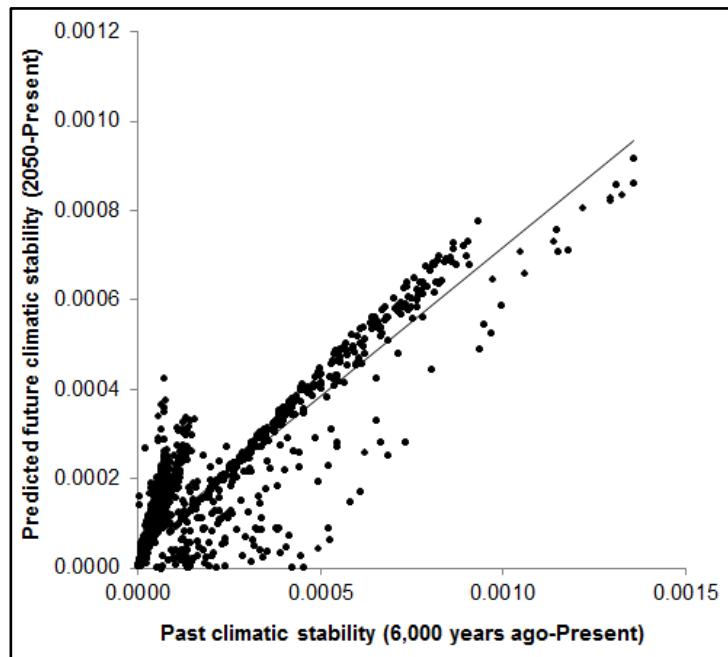


Figure 3.13. Relationship between past climatic stability (difference in relative occurrence rate between the mid-Holocene and present) and future climatic stability (difference in ROR between 2050 and present), as predicted by the highest performing Maxent model, Model A, at each geographical point where genetic distance was interpolated.

Finally, extracting the changes in suitability between future (2050, RCP2.6, Model A), and present at each of the interpolated genetic distance points and plotting these against longitude allowed me to quantitatively explore the relationship between changes in climatic suitability and geographical location. This plot reveals that the Cape Metropole, in the far west, is predicted to experience far greater losses in climatically suitable space in the future than the Overstrand, which is expected to experience gains in suitability by 2050 under RCP 2.6 (Figure E.1, Appendix E). When the same plot was generated using projections for 2050 under RCP8.5, the Overstrand also shows losses in climatically suitable space, but these losses were still of a lesser magnitude than those predicted to occur in the Cape Metropole (Figure E.2, Appendix E). These findings mirror the patterns observed in the suitability maps generated by the SDMs.

4. Discussion

4.1. Genetic differentiation across the species' distribution

The results of the population genetic analyses support the existence of two distinct genetic groups within *S. pantherina*, comprised of the populations of the Cape Metropole and Overstrand. This is in agreement with the previous population genetic study of the species (Measey & Tolley 2011). The presence of two haplotypes occurring in sample sites in both areas can be explained in two ways. Because dispersal across a distance of 100 km is highly unlikely for a single individual, these genetic variants could be ancestral forms from a time when the two groups were exchanging genes, or their presence could be an artefact of human-mediated movement between the two areas. The significantly higher haplotypic diversity identified in the populations of the Cape Metropole when compared to those in the Overstrand is in agreement with the previous study, and suggests that the persistence of the eastern populations may be in jeopardy, with historical breeding sites in Pringle Bay and Kleinmond already considered extinct. The discrepancy in diversity between the two areas could be due to a number of factors. Firstly, the west may be important in harbouring intraspecific diversity, either due to environmental heterogeneity and diversification in fragmented breeding ponds, or to the stable persistence of populations, allowing for the accumulation of genetic variants despite heavy urbanisation. This finding is in agreement with other studies which have highlighted the potential of urban wetlands as refugia for aquatic vertebrates, including amphibians, facilitating the persistence of diverse populations (Gledhill et al 2008). In fact, one study of three North Iberian amphibian species found that urban ponds harboured higher diversity in mitochondrial DNA than rural wetlands (Garcia-Gonzalez & Garcia-Vazquez 2012). Additionally, a study on the Australian lizard *Intellagama lesuerii* identified genetic divergence between populations in urban city parks, separated by less than five kilometres, with mitochondrial DNA divergence between parks 33 times higher than that seen between similarly distanced non-urban populations of the same species (Littleford-Colquhoun 2017). Furthermore, the study showed that this pattern is not due simply to genetic drift, but to adaptive diversification occurring in these park “archipelagos.” Taken together, this evidence supports the idea that relatively disconnected urban breeding sites, as experienced by *S. pantherina* in the Cape Metropole, can facilitate rapid local adaptation. Alternatively, the fact that the Overstrand is genetically depauperate compared to the Cape Metropole may suggest that the species is more sensitive to intensive agriculture, which may be causing population declines in that region.

The SAMOVA indicated that there was no genetic structure within the populations of the Cape Metropole or Overstrand, at least when compared with the level of differentiation between the two regions. However, there were some significant differences in levels of genetic diversity between samples sites within the population of the Cape Metropole. Examining these differences can give insight into the connectivity between breeding sites and can give an early indication that a particular site is experiencing a decline in numbers. Studies of habitat fragmentation have shown that the short-term impact of fragmentation increases as a function of the dispersal ability of a species (Cushman et al. 2005). Therefore, it follows that species that frequently move between breeding ponds will be most affected by factors that fragment habitat, such as urbanisation. This is especially worrying considering recent recognition that many amphibian populations are not structured as metapopulations, but rather experience relatively frequent dispersal, even when separated by kilometres (Smith & Green 2005). The fact that inter-pond dispersal in amphibians in undisturbed habitats, which many studies have found to be around 20% per generation, is quite widespread, suggests that lack of connectivity of ponds arising from habitat fragmentation should be a serious conservation concern (Marsh & Trenham 2001). Although there is still a relative lack of information on the movement dynamics of *S. pantherina*, preliminary data from radio-tracked individuals suggests that the species' average post-breeding dispersal distance is higher than that of other amphibians studied, with a maximum movement distance of over a kilometre reported, even in an agricultural landscape (Doucette-Riise 2012, Unpub. MSc. thesis).

On the contrary, my results suggest that this same propensity for movement is not demonstrated in an urban environment. The low levels of haplotype diversity in Observatory and Hout Bay, which were also reflected in the genetic landscapes (Figure 3.5b), suggest a lack of gene flow between these areas and surrounding breeding sites, and may indicate that these populations are declining. In the case of Observatory, the main breeding area is wedged between two major roadways. Although some individuals are likely able to cross these roadways, the wider urban landscape, characterized by multiple road networks and fences, likely presents a barrier to gene flow. Additional direct pressure from development projects in the area likely compounds the effect of reduced gene flow, causing a reduction in diversity. The high levels of genetic diversity identified in Zandvlei suggests that the currently established Zandvlei Estuary Reserve and associated watersheds is an important stronghold for the species, and is likely to be an important area in terms of preserving intraspecific diversity into the future. The high

genetic diversity at Kirstenhof could be the result of individuals moving into the area from Zandvlei, as there is a waterway connecting these two areas.

4.2. Predicted range shifts

The current distribution of the species predicted by the models shows a high probability of occurrence in the species' currently occupied area; however, it also predicts climatic suitability outside of the native range. This is partially due to the fact that the model can predict only the potentially suitable habitat for the species, and shows the species' fundamental, rather than its realized niche. The model's prediction that the area around the former breeding sites at Kleinmond and Pringle Bay is climatically suitable suggests that the disappearance of the toad from these localities is not related to changing climatic conditions, and is likely due to habitat loss and fragmentation associated with other anthropogenic pressures. The model's over-prediction is also a by-product of the fact that only a few climate-related variables were used to predict the species distribution, without consideration of other limiting factors such as biotic interactions, substrate, or land-use. Given the fact that much of the currently occupied habitat of *S. pantherina* is characterised by ongoing land transformation (urbanisation and agriculture), it is likely that the distribution projected here is optimistic, and future efforts to model the species' distribution should consider incorporating variables describing the magnitude of land-use change across the range. In addition, by including both breeding sites and foraging areas in the occurrence dataset used in SDM, it was assumed that the sum of both of these areas limits the species' distribution. However, it could be argued that one should use only records from breeding sites in building the SDM to reflect the fact that the climatic characteristics of foraging areas are relatively unimportant, and the species is primarily limited by availability of suitable breeding habitat at the larval stage. Using only records from breeding sites, a smaller subset of climate conditions associated with the breeding pond would inform the model of the climatic requirements of the species, and could potentially alter predictions of range shifts associated with climate change.

Projecting the SDMs into the future shows that most of the currently occupied area of *S. pantherina* will become climatically unsuitable by 2070, with the degree of suitability loss dependent on the both the greenhouse gas trajectory (RCP) and time period (2050 or 2070). Though this degree of suitability loss appears extreme, it seems more realistic considering projections of a 10–40% decline in winter rainfall in the Western Cape by this time (Engelbrecht et al. 2009). Regardless of the future time period or RCP, the projections suggest

that the Cape Peninsula and the rest of the currently occupied area in the Cape Metropole will be more affected by future climate change than the Overstrand, which is expected to experience gains in suitable habitat in some projections. This would suggest that any future efforts to mitigate the negative effects of climate change on the species should prioritise the populations of the Cape Metropole. That said, testing different combinations of variables as predictors in building the SDM revealed that variable selection can have a marked effect on the distributions projected by the model for both future and past time periods. Because the true set of environmental variables that determines the distribution of *S. pantherina* is unknown, the best strategy to select variables for use in the model was to consider the life history of the species together with variables that are generally considered important in limiting the ranges of other amphibian species. Although the models built with different combinations of predictors produced different projected distributions for the future, the pattern of higher losses in climatically suitable space in the current area of occurrence in the Cape Metropole was clear in the projections of both models.

The future suitability maps also indicate that some habitat outside of the native range will become climatically suitable in future. These areas may represent real suitable areas, that would be difficult for the species to colonize because of biogeographic barriers, or they could be the result of model projecting into un-sampled environmental space, or of incorrect predictor specification. If they are in fact suitable areas, whether or not the species is actually able to take advantage of these newly suitable areas depends on a number of factors. One of the most basic of these is, of course, the ability of the species to get to these new areas, which depends on its dispersal ability. Studies have shown that the success of Bufonid toads in colonizing the globe is owing to the independent evolution of lineage-specific traits that contribute to an ideal range-shifting phenotype (Van Bocxlaer et al. 2010). These include the presence of parotoid glands containing molecules that aid in water retention during the dry season; the presence of fat storage organs; cutaneous adaptations allowing adults to be un-reliant on the constant availability of a water body or moist substrate; and large size, as a small surface area to volume ratio equates to less water loss (Van Bocxlaer et al. 2010). As *S. pantherina* possesses these characteristics, it may be able to colonize new areas as its current area of occurrence becomes climatically unsuitable. Additionally, the speed with which other Bufonid toads, namely the cane toad *Rhinella marina*, are capable of evolving traits, such as long legs, that facilitate the rapid invasion of new areas, suggests that *S. pantherina* could evolve an even more optimal dispersal phenotype relatively quickly in response to the strong selection pressure posed by

rapid climate change (Phillips et al. 2006b). The previously mentioned long-distance dispersal capabilities, at least of some individuals, support the notion that the species has the potential to colonize some new areas as they become climatically suitable. Additionally, Model B predicts high RORs in interior montane regions. These areas are unlikely to be real areas of suitability for the species, and seem to be an artefact of the relationship with one of the environmental predictors, precipitation in the wettest month (BIO13), which shows a strong positive relationship with ROR.

While the results provide a good starting point from which to begin to formulate contingency plans for species in a changing climate, species distribution modelling, particularly this correlative method, carries a number of criticisms (reviewed by Pearson & Dawson 2003). One of the major weaknesses of the method is that, even with the best resolution climate data available, it is impossible to capture microhabitat conditions. This is especially problematic given the proven ability of microhabitats to decrease the vulnerability of communities in the face of extreme climatic events, likely reducing mortality and enabling species persistence in otherwise climatically unsuitable areas (Scheffers et al. 2014). This is particularly important in the case of amphibian species, for which microhabitats have been shown to affect body temperature and dehydration rates (Seebacher & Alford 2002). Another weakness of the method is that it fails to take into account a number of factors, such as biotic interactions, which limit the species' distribution in the natural environment (Davis et al. 1998). Another variable which may be important in determining species' distributions is soil quality, which may include texture, moisture, and pH. Both moisture and pH have been found to influence amphibian distributions, and pH has been shown to affect reproductive success in the Cape platanna, another species that occurs in the south-western Cape (Wyman 1988; Picker 1993). This suggests that substrate pH may be a factor limiting the distribution of *S. pantherina*. Another important, yet often excluded, variable is land cover. Future studies of the impact of climate change on species distributions, particularly in species like *S. pantherina* which occur in heavily urbanized and agricultural settings, should consider spatial variation in the fraction of untransformed habitat. Finally, correlative species distribution modelling ignores the potentially rescuing impact of plasticity and adaptation. The entire discipline of population genetics is founded around the principle that species are not uniform entities, highlighting the importance of incorporating genetic differentiation as an element in species distribution modelling. Indeed, it has been shown that incorporating population differentiation into species distribution models can affect projected range shifts (Valladares et al. 2014). These limitations

are important to keep in mind when interpreting the results of the SDMs, particularly when trying to project range shifts under climate change scenarios. That said, considering the rapid acceleration of climate change we currently face, together with the range contractions projected by multiple models, it is important to take action despite the uncertainty.

4.3. Potential impact of climate change on genetic diversity

Returning to the hypotheses posed at the outset regarding the potential impact of climatic changes on genetic diversity within the species, there was a significant relationship identified between the interpolated genetic distances and changes in climatic suitability between the mid-Holocene and present, where areas showing the greatest changes in suitability between past and present were of relatively high diversity. Those areas predicted to experience the greatest losses in climatic suitability in future had relatively high interpolated genetic diversities, and those areas predicted to experience the greatest gains in suitability also showed relatively high interpolated genetic diversities. The former corresponds to the populations of the Cape Metropole, while the latter corresponds to the populations of the Overstrand, which had generally higher interpolated genetic distances. This, paired with the significant negative correlation between the degree of past change in climatic suitability and interpolated genetic distance, supports the hypothesis that diversification in the species is driven by climatic fluctuations (i.e. that instability is the driver of diversification). The fact that interpolated genetic distances were generally higher in the Overstrand is likely a result of the method used to generate the genetic landscapes, where the values are interpolated between sample sites, and suggests that there is less gene flow, in general, and higher genetic distances between the sample sites used as input in generating the landscape.

The trend identified between the nucleotide and haplotype diversity values for the sample sites suggests the same pattern of higher diversity in areas of generally higher losses in climatically suitable space. Generally higher losses in climatically suitable space in the more diverse sites of the Cape Metropole, coupled with significantly higher nucleotide diversity in areas of relatively high change in climatic suitability between the mid-Holocene and present supports the hypothesis that climatic instability, rather than stability, drives diversification. Because there were only 13 sample sites, the statistical power for picking up a relationship between haplotype diversity and changes in suitability was inherently low. If more sample sites were available, a significant relationship between haplotype diversity and changes in suitability may be identified, mirroring that identified when looking at nucleotide diversity.

Although the interpolated genetic landscape does provide a large sample for identifying patterns, this approach has limitations. The interpolated genetic distance values are not statistically independent of one another, since they are interpolated from a relatively small number of presence points. Therefore, the high statistical power afforded by this large number of interpolated values is illusory and should be interpreted with caution. Due to the limitations associated with using the genetic landscape approach, the actual, empirically estimated diversity at each of the sample sites is more informative, both for evaluating the hypotheses and, in a practical conservation context, for identifying the sample sites most at risk of losing climatically suitable habitat with climate change. Importantly, all models looking at the interpolated genetic distances, haplotype diversity, and nucleotide diversity values at the sample sites agreed that there would be higher predicted losses in climatic suitability in the Cape Metropole populations, when compared with the Overstrand populations, suggesting that climate change mitigation efforts should prioritise the western part of the species' distribution.

In general, the relationships identified between the genetic diversity metrics and changes in climatic suitability through time were robust to changes in the Model, future time period, or RCP used in analyses. Although these factors did influence model fit in some cases, the same trends are apparent. In general, the percentage of the deviance in genetic diversity explained by the models, particularly in the linear model where haplotype diversity was used as the response variable, was low, indicating that other, non-climate-related, variables are important in explaining the variation in the diversity data. Although these missing predictors are unknown, variables characterizing levels of habitat fragmentation may be important in explaining the observed variation in diversity, as isolated sample sites are likely to harbour lower genetic diversity.

The potential relationship between genetic diversity and climate-induced habitat loss was examined here in order to assess whether populations that harbour valuable genetic diversity, and are thus important for the long-term persistence of the species, will be lost with climate change. Because genetic diversity was considered in this context as a proxy for adaptive potential, the use of a neutral genetic marker is a limitation. The use of non-coding markers in exploring the ability of species to respond to environmental change has been criticised, as variation in neutral markers does not always reflect quantitative genetic variation (Reed & Frankham 2001; Knopp et al 2007). However, a number of studies do seem to suggest a positive correlation between the two indices, suggesting that variation in neutral markers can act as a

rough predictor of levels of variation in coding regions (Leinonen et al. 2008; Merilä & Crnokrak 2001).

4.4. The relationship between climatic stability and genetic diversity

A broader aim of this study was to test predictions of the hypothesis that historical climatic instability is a mechanism driving diversification, which may explain an overlap between areas of high diversity and high risk of change in habitat suitability with future predicted climate change. This hypothesis assumes that levels of past climatic stability are predictive of future climatic stability in an area. The strong linear relationship identified here between past climatic stability and future climatic stability in an area satisfies this condition, therefore the hypothesis that climatic stability is driving diversification cannot be rejected. However, correlation does not imply causation, and it is important to note that other factors unrelated to climate are likely to contribute to patterns of genetic diversity across a species' range. For example, historical fluctuations in effective population size may have resulted in inbreeding and loss of genetic diversity. Additionally, current landscape attributes, such as land transformation and habitat fragmentation, may be drivers of variation in levels of genetic diversity across the landscape. It is likely that land transformation influences patterns of diversity in the case of *S. pantherina*, as the species faces habitat alteration from urbanisation and agriculture, respectively, in its areas of occurrence in the Cape Metropole and the Overstrand. If demographic processes are more robust to urbanisation, than to agriculture, this may be an additional, non-climate related factor that might explain the higher diversity observed in the Cape Metropole. The recent extinction of the former populations at Betty's Bay, Pringle Bay, and Kleinmond in the Overstrand region suggest that this may be the case, and future work should address the influence of matrix characteristics on demographic parameters and population persistence. Furthermore, the analysis presented here could be extended to evaluate this alternative hypothesis by using a model to investigate the relative magnitude of the associations between genetic diversity and a) a measure of matrix quality or degree of land transformation and b) climatic stability through time.

Another important consideration when evaluating a potential relationship between genetic diversity and historical climatic stability is that the nature of this relationship is likely dependent on the temporal scale over which climatic stability is examined. Here, the period from the mid-Holocene to present day was used as a snapshot of the divergence history of the species, as it is known that diversification between the two areas of the distribution occurred

relatively recently, suggesting that further diversification within the two groups is likely to have happened relatively recently as well. This limits the inferences that can be made from the data presented here, and future studies aimed at addressing this hypothesis should model multiple historical time periods in relation to genetic diversity to determine how robust the relationship is to changes in the temporal scale examined.

In the case of *S. pantherina*, only a small number of sample sites ($n = 13$) was available for analyses. Although some significant relationships were identified using this sample size, the interpolated genetic distances values are likely the only dataset of sufficient size to detect all significant relationships between diversity metrics and changes in climatic suitability. This forces one to either make an interpretation based on a large number of interpolated values, which are themselves derived from a mathematical model and do not represent actual measured diversity values, or make an interpretation based on only 13 values. Further studies of a similar nature on multiple species, preferably for which there are more than 13 sample sites and, which have a more continuous distribution, are necessary to more thoroughly test this hypothesis in nature. Multi-species studies of a similar nature replicated in different regions of the globe would be required to explore the generality of the hypothesis, its ability to explain global patterns of diversity, and to identify whether there is a positive relationship between areas of high risk of losing climatically suitable space with future predicted climate change and genetic diversity on a larger scale.

4.5. Conservation implications

The distinct genetic composition of the two areas supports their treatment as separate management units, as each group harbours unique genetic variants not found in the other. Among the populations in the Cape Metropole, Observatory and Hout Bay were significantly lower in diversity than other sites. Therefore, efforts should be made to increase the connectivity within the matrix between sample sites in order to allow movement of individuals and genetic rescue for these low diversity populations. This might be done by means of a built corridor allowing toads to safely cross major roadways adjacent to breeding sites and/or by increasing the quality of the matrix between breeding sites through stepping stones of artificial wetlands and urban gardens, which have been commonly observed to support leopard toads. Built corridors such as pipe culverts, or concrete or metal tunnels used to vary water beneath roads have been used successfully to allow movement of wildlife, including amphibians, across roads (Glista et al. 2009).

In order to mitigate the negative effects of climate change on breeding sites, engineered solutions may be the only option. Though efforts of this type are poorly tested, they hold promise for promoting amphibian population persistence under uncertain climate conditions. These may include the creation of microhabitat refuges, restoration of breeding sites, and hydrological manipulation of existing breeding sites (Shoo et al. 2011). Creating new, and hydrologically diverse, wetlands can be effective at preventing the extinction of breeding sites in threatened amphibian species (Rannap et al. 2009). These strategies may be effective in preventing the further loss of breeding sites of *S. pantherina*.

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Appendices

Appendix A

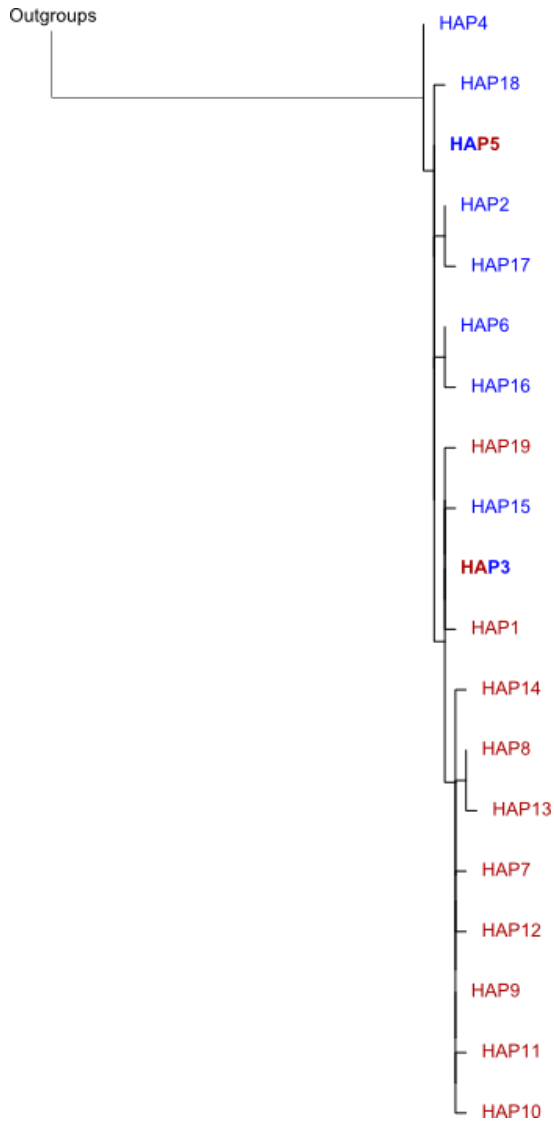


Figure A.1. Maximum likelihood (ML) phylogenetic tree showing the relationship between mitochondrial ND2 haplotypes of *Sclerophrys pantherina*. Outgroups (not shown) include *Sclerophrys rangeri* and *Sclerophrys garmani*. Haplotypes in red only occur in sample sites of the Cape Metropole, those in blue only in the Overstrand, and those in both colours are common between the two areas.

Appendix B

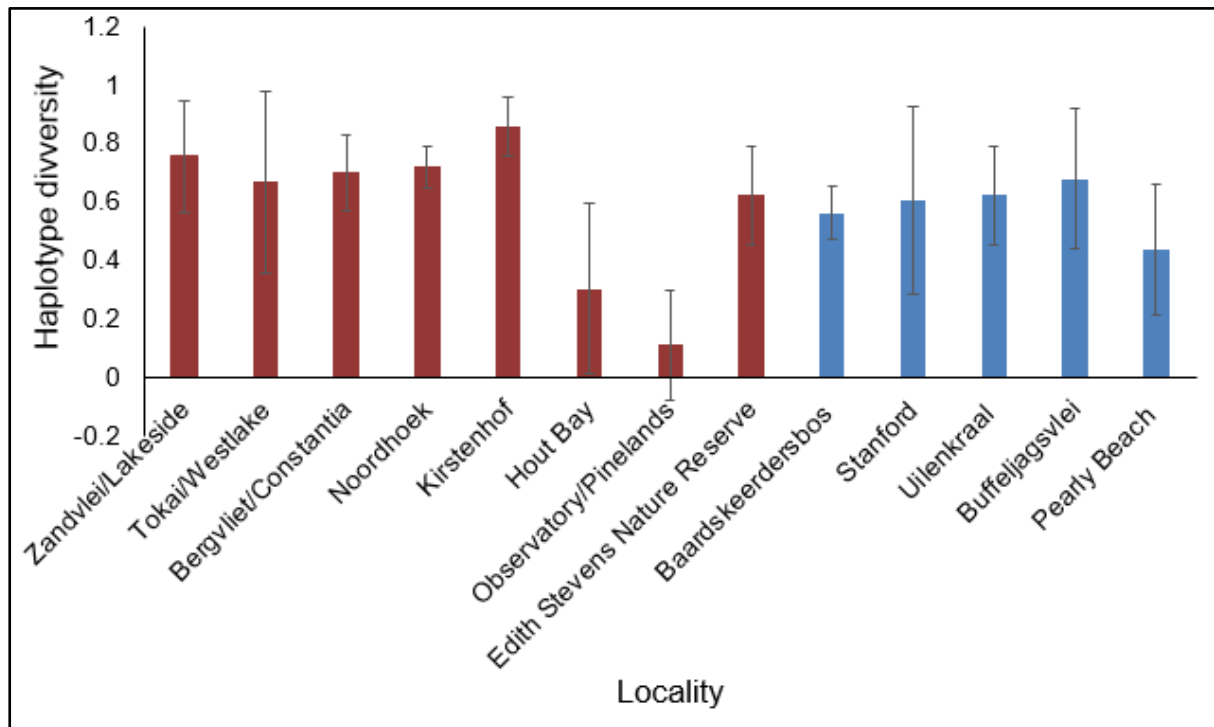


Figure B.1. Haplotype diversity value for each locality calculated from mitochondrial ND2 sequences of *Sclerophrys pantherina*, with error bars showing the 95% confidence intervals around the mean. Red bars represent localities in the Cape Metropole, and blue bars represent localities in the Overstrand.

Appendix C

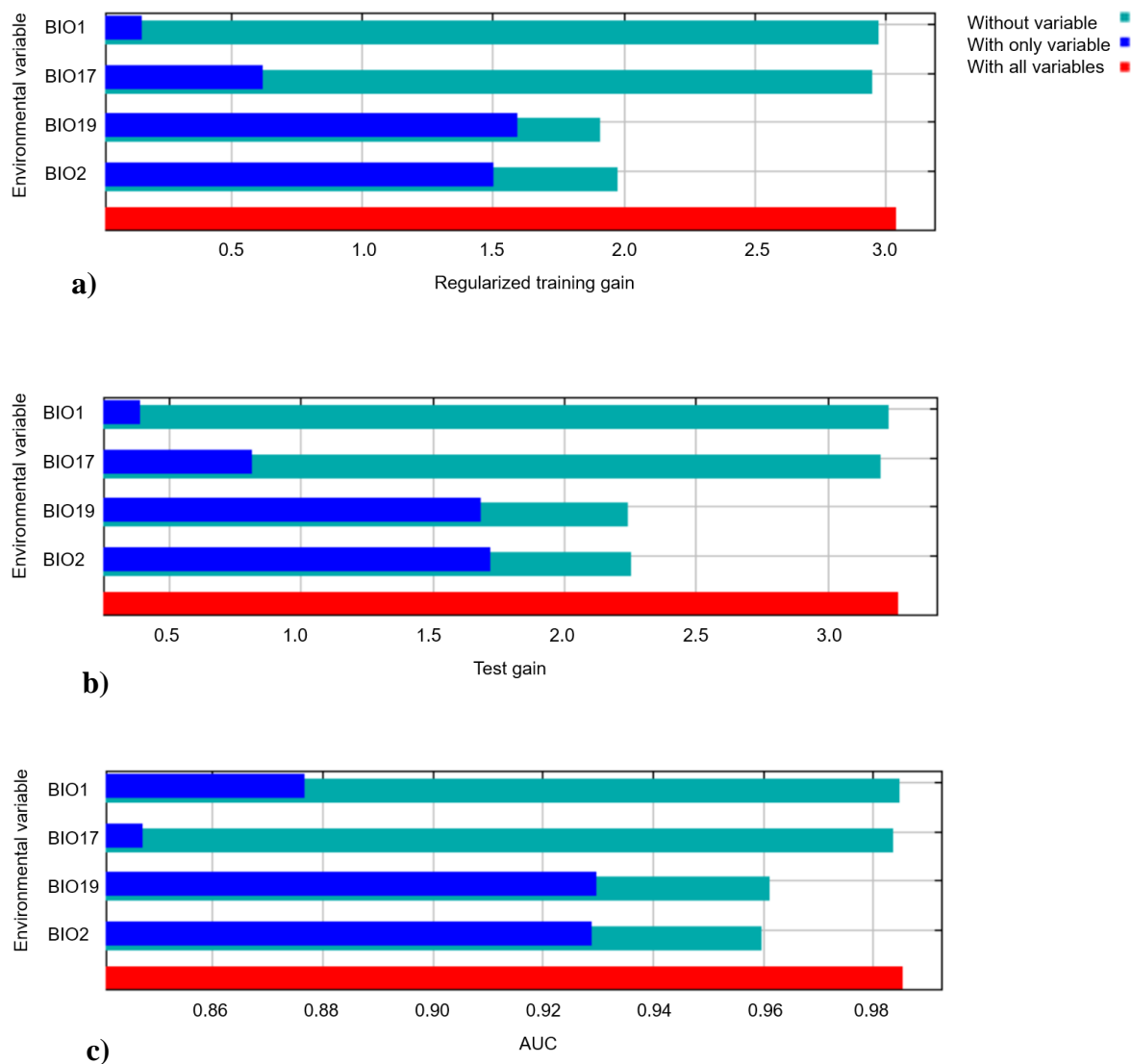
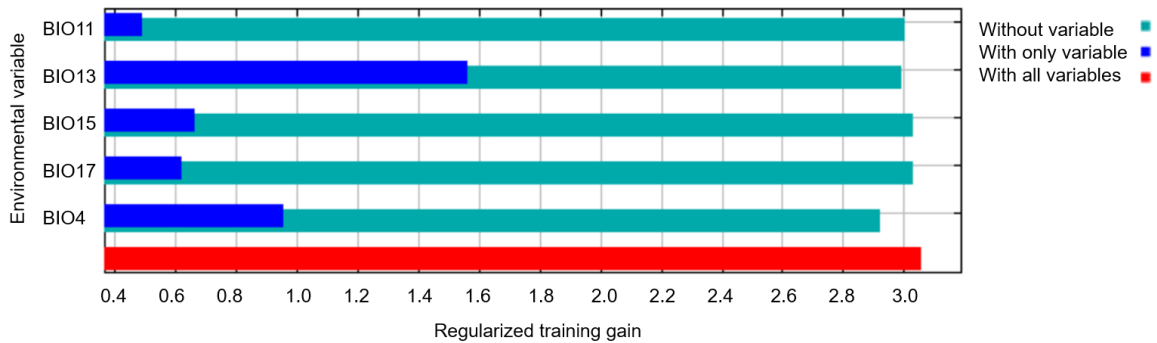
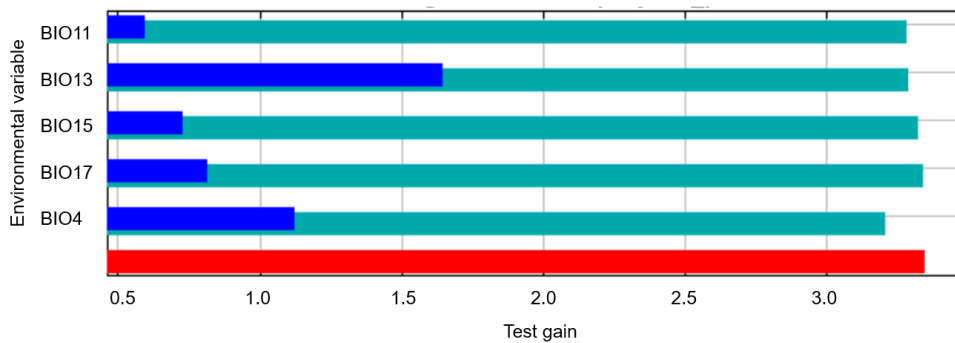


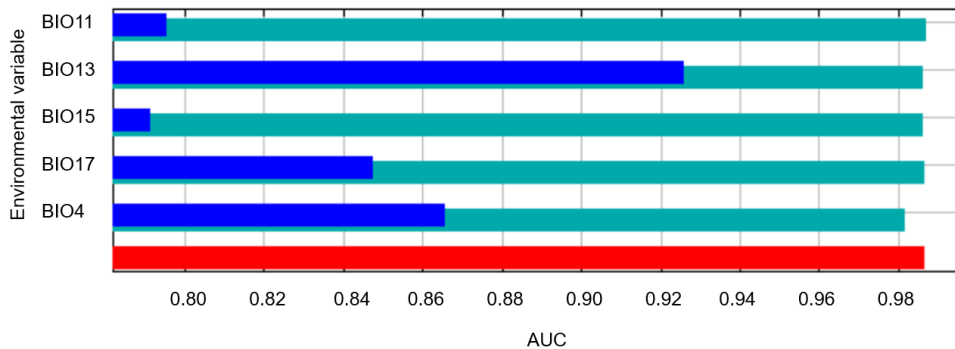
Figure C.1. Results of jack-knife resampling tests used to determine the importance of individual variables in building Minxent **Model A** (the highest performing model). This test works by running the model multiple times, excluding one variable each time, and measuring the resulting reduction in model fit, as measured by regularized training gain (a), test gain (b), and AUC (c). The model is then created with each variable on its own, and model fit is evaluated in the same way. BIO1= Annual mean temperature, BIO2= Mean diurnal temperature range, BIO17= Precipitation of the driest quarter, BIO19= Precipitation of the coldest quarter.



a)



b)



c)

Figure C.2. Results of jack-knife resampling tests used to determine the importance of individual variables in building Minxent **Model B** (built from an alternative set of bioclimatic variables assumed to be relevant to the species' ecology). This test works by running the model multiple times, excluding one variable each time, and measuring the resulting reduction in model fit, as measured by regularized training gain (a), test gain (b), and AUC (c). The model is then created with each variable on its own, and model fit is evaluated in the same way. BIO4= Temperature seasonality, BIO11= Mean temperature of the coldest quarter, BIO13= Precipitation of the wettest month, BIO15= Precipitation seasonality (coefficient of variation), BIO17= Precipitation of the driest quarter.

Appendix D

Table D.1. Results of statistical models used to test the hypothesis that climatic stability drives diversification, using the projections of Minxent **Model B** (built from an alternative set of bioclimatic variables assumed to be relevant to the species' ecology). Results of linear regressions with nucleotide and haplotype diversity values for the 13 sample sites as the response variable, and the difference in climatic suitability (ROR) between the mid-Holocene and present as the predictor variable are shown in the unshaded cells. Regression statistics shown include the regression coefficient (Coeff) and its standard error (SE coeff), the t-value (t), p-value (p), adjusted R², and the percentage of deviation explained by each model (Dev.exp. %). Results of a general additive model (GAM) with interpolated genetic distance as the response variable and the difference in climatic suitability (ROR) between the mid-Holocene and present as the predictor variable are given in the shaded cells. "Region" (Cape Metropole or Overstrand) was incorporated as fixed effect in the GAM, and was highly significant predictor of genetic diversity (p<0.001). GAM results include the degrees of freedom (df), the F-statistic (F), p-value (p), as well as the adjusted R² and the percentage of deviation explained by each model (Dev. Exp. %).

Response var.	df	Coeff.	SE (coeff.)	F/t	p	R ² (adj.)	Dev. exp. (%)
Nucleotide div	11	-0.896	0.400	-2.24	0.047	0.251	31.3
Haplotype div	11	-217	186	-1.17	0.269	0.029	11
Interpolated div	4	-	-	362	<0.001	0.566	56.6

Table D.2. Results of general additive models (GAMs), with interpolated genetic distance as the response variable and the difference between future time periods and present (for either 2050 or 2070, RCP 2.6 or RCP8.5, Model A or Model B) as the predictor variable. Model A refers to the highest performing Minxent model, while Model B refers to a model built from an alternative set of bioclimatic variables assumed to be relevant to the species' ecology. "Region" was incorporated as a fixed effect and was a highly significant predictor of genetic diversity (p<0.001). GAM results include the F-statistic (F), p-value (p), as well as the adjusted R² and the percentage of deviation explained by each model (Dev. Exp. %). Degrees of freedom was equal to 4 for all models.

Projection used	F	p	R ² (adj.)	Dev. exp. (%)
Model A, 2050 RCP8.5	886.6	<0.001	0.608	60.9
Model A, 2070 RCP2.6	883.5	<0.001	0.608	60.8
Model A, 2070 RCP8.5	881.9	<0.001	0.608	60.8
Model B, 2050 RCP2.6	382.0	<0.001	0.568	56.8
Model B, 2050 RCP8.5	450.5	<0.001	0.574	57.4
Model B, 2070 RCP2.6	434.9	<0.001	0.573	57.3
Model B, 2070 RCP8.5	448.4	<0.001	0.574	57.4

Table D.3. Results of linear regressions with **haplotype** diversity values for the 13 sample sites as the response variable and the difference between future time periods and present (for either 2050 or 2070, RCP 2.6 or RCP8.5, Model A or Model B) as the predictor variable. Model A refers to the highest performing mident model, while Model B refers to a model built from an alternative set of bioclimatic variables assumed to be relevant to the species' ecology. Regression statistics shown include the regression coefficient (Coeff.) and its standard error (SE coeff.), the t-value (t), p-value (p), adjusted R², and the percentage of deviation explained by each model (Dev. exp %). Degrees of freedom was equal to 11 for both models.

Projection used	Coeff.	SE (coeff.)	t	p	R ² (adj.)	Dev. exp. (%)
Model A, 2050 RCP8.5	-280	195	-1.44	0.178	0.082	15.8
Model A, 2070 RCP2.6	-210	171	-1.23	0.244	0.041	12.1
Model A, 2070 RCP8.5	-278	194	-1.43	0.180	0.081	15.7
Model B, 2050 RCP2.6	-251	205	-1.23	0.245	0.041	12.1
Model B, 2050 RCP8.5	-274	205	-1.34	0.208	0.062	14.0
Model B, 2070 RCP2.6	-254	198	-1.28	0.227	0.051	13.0
Model B, 2070 RCP8.5	-271	204	-1.33	0.210	0.061	13.9

Table D.4. Results of linear regressions with **nucleotide** diversity values for the 13 sample sites as the response variable and the difference between future time periods and present (for either 2050 or 2070, RCP 2.6 or RCP8.5, Model A or Model B) as the predictor variable. Model A refers to the highest performing Minxent model, while Model B refers to a model built from an alternative set of bioclimatic variables assumed to be relevant to the species' ecology. Regression statistics shown include the regression coefficient (Coeff.) and its standard error (SE coeff.), the t-value (t), p-value (p), adjusted R², and the percentage of deviation explained by each model (Dev. exp %). Degrees of freedom was equal to 11 for both models.

Projection used	Coeff.	SE (coeff.)	t	p	R ² (adj.)	Dev. exp. (%)
Model A, 2050 RCP8.5	-1.051	0.411	-2.56	0.026	0.317	37.3
Model A, 2070 RCP2.6	-0.842	0.366	-2.30	0.042	0.263	32.4
Model A, 2070 RCP8.5	-1.047	0.410	-2.56	0.027	0.316	37.3
Model B, 2050 RCP2.6	-1.019	0.436	-2.34	0.040	0.271	33.1
Model B, 2050 RCP8.5	-1.062	0.434	-2.45	0.032	0.293	35.2
Model B, 2070 RCP2.6	-1.005	0.422	-2.39	0.036	0.281	34.1
Model B, 2070 RCP8.5	-1.056	0.433	-2.44	0.033	0.292	35.1

Appendix E

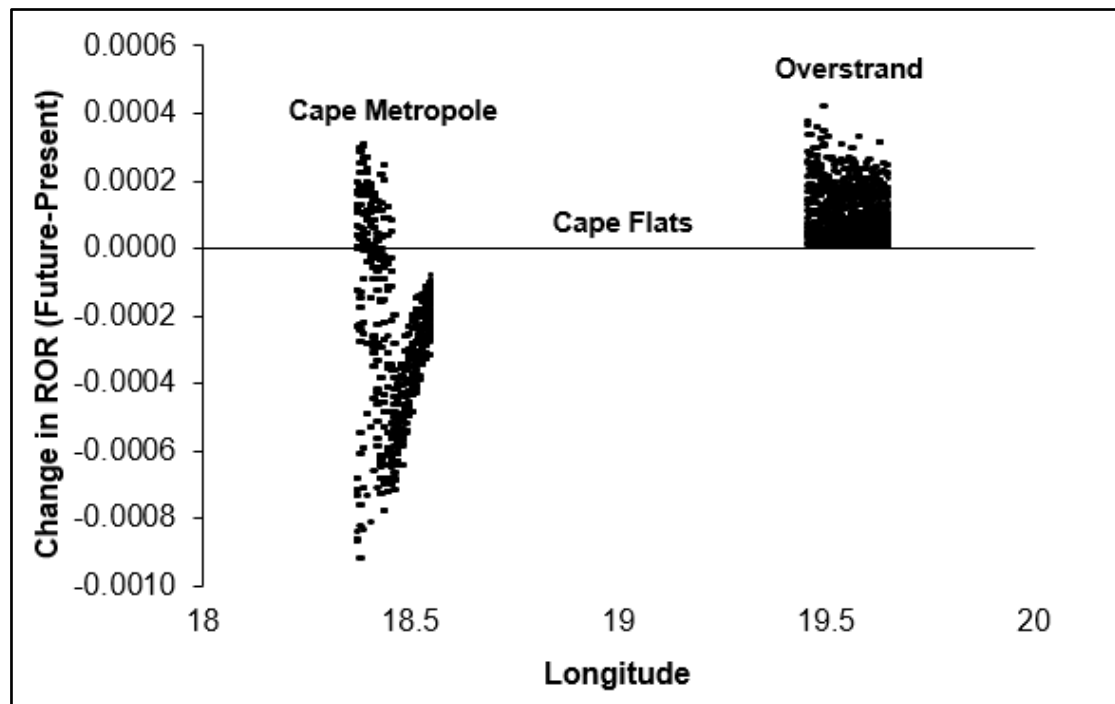


Figure E.1. Change in ROR (i.e. climatic suitability) between future (2050, Minxent Model A- the highest performing model) at each point where an interpolated genetic distance was calculated, using projections for **RCP2.6, the “best-case” greenhouse gas trajectory**. Interpolated landscapes were created for the Cape Metropole and Overstrand separately, due to the disjunct distribution across the Cape Flats. This suggests that the Cape Metropole will generally experience losses in climatically suitable space, while the Overstrand will experience substantial gains.

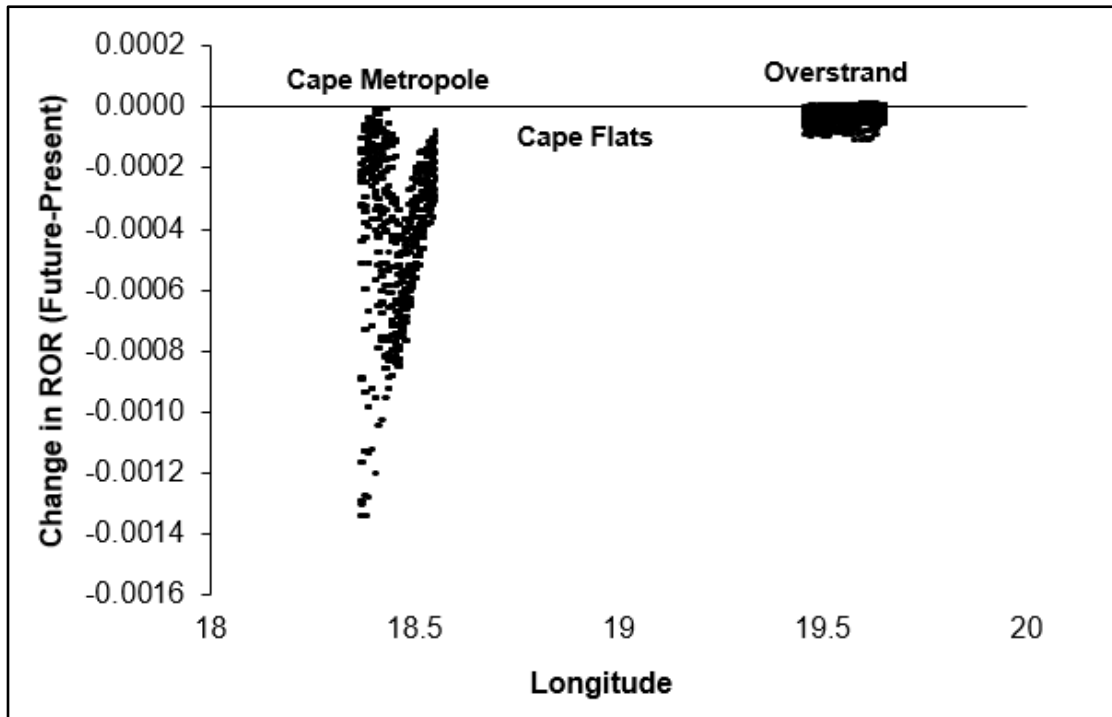


Figure E.2. Change in ROR (i.e. climatic suitability) between future (2050, Minxent Model A- the highest performing model) at each point where an interpolated genetic distance was calculated, using projections for **RCP8.5, the “worst-case” greenhouse gas trajectory**. Interpolated landscapes were created for the Cape Metropole and Overstrand separately, due to the disjunct distribution across the Cape Flats. Under this scenario, both regions are predicted to experience losses in suitability with future projected climate change, with the Cape Metropole showing the greatest magnitude of suitability change between time periods.